This homologous gene is then isolated, expressed, and used as a target for candidate antibiotic compounds. In another aspect of this embodiment, the homologous gene (for example a homologous coding nucleic acid) thus identified, or a portion thereof, is transcribed in an autologous cell or microorganism or in a heterologous cell or microorganism in an antisense orientation in such a way as to alter the level or activity of a homologous gene required for proliferation in the autologous or heterologous cell or microorganism. Alternatively, a homologous antisense nucleic acid may be transcribed in an autologous or heterologous cell or microorganism in such a way as to alter the level or activity of a gene product required for proliferation in the autologous or heterologous cell or microorganism.

The nucleic acids homologous to the genes required for the proliferation of *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* or the sequences complementary thereto may be used to identify homologous coding nucleic acids or homologous antisense nucleic acids from cells or microorganisms other than *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* to inhibit the proliferation of cells or microorganisms other than

Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or *Yersinia pestis* by inhibiting the activity or reducing the amount of the identified homologous coding nucleic acid or homologous polypeptide in the cell or microorganism other than *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* or to identify compounds which inhibit the growth of cells or microorganisms other than *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus*

mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or *Yersinia pestis* as described below. For example, the nucleic acids homologous to proliferation-required genes from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* or the sequences complementary thereto may be used to identify compounds which inhibit the growth of *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,*

Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the nucleic acids homologous to proliferation-required sequences from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* (including nucleic acids homologous to one of SEQ ID NOs.: 6214-42397) or the sequences complementary thereto (including nucleic acids homologous to one of SEQ ID NOs.: 1-6213) are used to identify proliferation-required sequences in an organism other than *E. coli.*

In another embodiment of the present invention, antisense nucleic acids complementary to the sequences identified as required for proliferation or portions thereof (including antisense nucleic acids comprising a nucleotide sequence complementary to one of SEQ ID NOs.: 6214-42397 or portions thereof, such as the nucleic acids of SEQ ID NOs.: 1-6213) are transferred to vectors capable of function within a species other than the species from which the sequences were obtained. For example, the vector may be functional in *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,*

Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the vector may be functional in an organism other than *E. coli*. As would be appreciated by one of ordinary skill in the art, vectors may contain certain elements that are species specific. These elements can include promoter sequences, operator sequences, repressor genes, origins of replication, ribosomal binding sequences, termination sequences, and others. To use the antisense nucleic acids, one of ordinary skill in the art would know to use standard molecular biology techniques to isolate vectors containing the sequences of interest from cultured bacterial cells, isolate and purify those sequences, and subclone those sequences into a vector adapted for use in the species of bacteria to be screened.

Vectors for a variety of other species are known in the art. For example, numerous vectors which function in *E. coli* are known in the art. Also, Pla et al. have reported an expression vector that is functional in a number of relevant hosts including: *Salmonella typhimurium, Pseudomonas putida, and Pseudomonas aeruginosa. J. Bacteriol.* **172**(8):4448-55 (1990). Brunschwig and Darzins (Gene (1992) 111:35-4, described a shuttle expression vector for *Pseudomonas aeruginosa.* Vectors useful for the production of stabilized mRNA having an increased lifetime (including antisense RNA) in Gram negative organisms are described in U.S. Provisional Patent Application Serial Number 60/343,512, filed December 21, 2001. Similarly many examples exist of expression vectors that are freely transferable among various Gram positive microorganisms. Expression vectors for *Enterococcus faecalis* may be engineered by incorporating suitable promoters into a pAK80 backbone (Israelsen, H., S. M. Madsen, A. Vrang, E. B. Hansen and E. Johansen. 1995. *Appl. Environ. Microbiol.* **61**:2540-2547. A number of vectors useful for nucleic acid expression (including antisense nucleic acid expression) in *Enterococcus faecalis, Staphylococcus areus* as well as other Gram positive organisms are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001.

Following the subcloning of the antisense nucleic acids complementary to proliferation-required sequences from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,*

Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or *Yersinia pestis* or portions thereof into a vector functional in a second cell or microorganism of interest (i.e. a cell or microorganism other than the one from which the identified nucleic acids were obtained), the antisense nucleic acids are conditionally transcribed to test for bacterial growth inhibition. The nucleotide sequences of the nucleic acids from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* that, when transcribed, inhibit growth of the second cell or microorganism are compared to the known genomic sequence of the second cell or microorganism to identify the homologous gene from the second organism. If the homologous sequence from the second cell or microorganism is not known, it may be identified and isolated by hybridization to the proliferation-required *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio*

cholerae or *Yersinia pestis* sequence of interest or by amplification using PCR primers based on the proliferation-required nucleotide sequence of interest as described above. In this way, sequences which may be required for the proliferation of the second cell or microorganism may be identified. For example, the second microorganism may be *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis* or any species falling within the genera of any of the above species. In some embodiments of the present invention, the second microorganism is an organism other than E. coli.

The homologous nucleic acid sequences from the second cell or microorganism which are identified as described above may then be operably linked to a promoter, such as an inducible promoter, in an antisense orientation and introduced into the second cell or microorganism. The techniques described herein for identifying *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus*

mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or *Yersinia pestis* genes required for proliferation may thus be employed to determine whether the identified nucleotide sequences from a second cell or microorganism inhibit the proliferation of the second cell or microorganism. For example, the second microorganism may be *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis* or any species falling within the genera of any of the above species. In some embodiments of the present invention, the second microorganism may be an organism other than E. coli.

Antisense nucleic acids required for the proliferation of microorganisms other than *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium*

tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or *Yersinia pestis* or the genes corresponding thereto, may also be hybridized to a microarray containing the *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* (including the nucleic acids of SEQ ID NOs.: 6214-42397) to gauge the homology between the *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* sequences and the proliferation-required nucleic acids from other cells or microorganisms. For example, the proliferation-required nucleic acid may be from *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida*

guilliermondii, Candida krusei, Candida kefyr (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis* or any species falling within the genera of any of the above species. In some embodiments of the present invention, the proliferation-required nucleotide sequences from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae* or *Yersinia pestis* or homologous nucleic acids are used to identify proliferation-required sequences in an organism other than E. coli. In some embodiments of the present invention, the proliferation-required sequences may be from an organism other than E. coli. The proliferation-required nucleic acids from a cell or microorganism other than *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae,*

Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or *Yersinia pestis* may be hybridized to the array under a variety of conditions which permit hybridization to occur when the probe has different levels of homology to the nucleotide sequence on the microarray. This would provide an indication of homology across the cells or microorganisms as well as clues to other possible essential genes in these cells or microorganisms.

In some embodiments of the present invention, the essential gene products described herein are used in methods of identifying a target on which a compound that inhibits cellular proliferation acts. Such methods are described in the U.S. Patent Application entitled METHODS FOR IDENTIFYING THE TARGET OF A COMPOUND WHICH INHIBITS CELLULAR PROLIFERATION, filed February 8, 2002. As employed herein, some embodiments of methods used to identify a target on which a compound that inhibits cellular proliferation acts utilize collections or cultures of strains comprising strains which either overexpress a different gene product which is required for cellular proliferation (such as the gene products described herein) or underexpress a different gene product (such as the gene products described herein) which is required for cellular proliferation (i.e. at least some of the strains in the culture overexpress or underexpress a gene product required for cellular proliferation). In some embodiments, the present invention uses collections or cultures of strains comprising both strains which overexpress gene products required for cellular proliferation and strains which underexpress the same gene products required for cellular proliferation. Preferably, each of the strains present in the culture or collection either overexpresses or underexpresses a different gene product which is required for cellular proliferation (i.e. all of the strains in the culture overexpress or underexpress a gene product required for cellular proliferation). However, in some embodiments, the culture or collection may include one or more strains which do not overexpress or underexpress a gene product which is required for proliferation. The gene product which is overexpressed or underexpressed in each strain may be any gene product which is required for cellular prolifereation, including a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous

antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene produet comprising a homologous polypeptide.

As used herein the term "culture" refers to a plurality of strains growing in a single aliquot of a liquid growth medium and the term "collection" refers to a plurality of strains each of which is growing in a separate aliquot of liquid growth medium or a different location on a solid growth medium.

In some embodiments, if desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product described herein which is required for cellular proliferation. In this embodiment, the gene products which are overexpressed or underexpressed in one or more of the strains may be functionally related or functionally unrelated. This may facilitate the identification of compounds when two or more gene products share similar functions in the cell or where the cell has multiple biochemical pathways which lead to a particular end product.

Alternatively, if the gene product described herein to be overexpressed or underexpressed is encoded by a gene which is part of an operon containing a plurality of genes, the desired gene may be overexpressed or underexpressed while the remaining genes in the operon are expressed at levels where they do not impact the ability of the cell to grow in the presence of a particular compound. For example, the desired gene may be placed under the control of a regulatable promoter, a transcriptional terminator may be placed 3' of the desired gene and a promoter, preferably a constitutive promoter, may be placed 3' of the transcriptional terminator and 5' of the remaining genes in the operon.

In some embodiments, the culture or collection of strains may comprise a strain which overexpresses or underexpresses a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least two gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 10 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 20 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 30 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 50 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 100 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 300 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213 or more than 300 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, wherein each strain in the culture or collection of strains overexpresses or underexpresses a single gene

product whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213. Alternatively, if desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213.

In other embodiments, the culture or collection of strains may comprise a strain which overexpresses or underexpresses a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least two gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 10 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 20 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 30 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 50 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 100 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 300 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 or more than 300 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, wherein each strain in the culture or collection of strains overexpresses or underexpresses a single gene product encoded by a nucleic acid selected from the group consisting of SEQ ID NOs. 6214-42397. Alternatively, if desired, one or more strains in the culture or collection of strains may overexpress or underexpress more than one gene product encoded by a nucleic acid selected from the group consisting of SEQ ID NOs. 6214-42397.

In some embodiments the culture or collection of strains comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least two gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 10 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 20 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 30 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 50 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 100 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 300 gene

products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 or more than 300 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, wherein each strain in the culture or collection of strains overexpresses or underexpresses a single gene product selected from the group consisting of SEQ ID NOs. 42938-78581. Alternatively, if desired one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product selected from the group consisting of SEQ ID NOs. 42938-78581.

In other embodiments, the culture or collection of strains comprises a strain in which at least one of the gene products encoded by a homologous coding nucleic acid as defined above is overexpressed or underexpressed. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300 or more than 300 gene products encoded by a homologous coding nucleic acid as defined above. If desired the culture or collection of strains may comprise one or more strains which overexpress or underexpress more than one gene product encoded by a homologous coding nucleic acid. In further embodiments, the culture or collection of strains comprises a strain in which at least one, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300 or more than 300 homologous polypeptides as defined above is overexpressed or underexpressed. If desired the culture or collection of strains may comprise one or more strains which overexpress or underexpress more than one homologous polypeptide.

For example, in some embodiments, the culture or collection of strains comprises a strain in which at least one gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product. In some

embodiments, the culture or collection of strains may comprise strains in which in aggregate at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300, or more than 300 gene products selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product.

If desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213.

In further embodiments, the culture or collection of strains comprises a strain in which at least one gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product. In some embodiments, the culture or collection of strains comprises a strain or a group of strains in which in aggregate at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300, or more than 300 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product.

If desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

In additional embodiments, the culture or collection of strains comprises a strain in which at least one gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product. In some embodiments, the culture or collection of strains comprises a strain or a group of strains in which in aggregate at least 2, at least 10, at least 20, at

least 30, at least 50, at least 100, at least 300, or more than 300 gene products comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product.

If desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581.

The methods of the present invention may be used to identify the targets of compounds which inhibit the proliferation of any desired cell or organism. In some embodiments, these methods are employed to identify the targets of compounds which inhibit the proliferation of bacteria, fungi, or protozoans. In further embodiments, these methods are employed to identify the targets of compounds which inhibit the growth of an organism selected from the group consisting of *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis* or any species falling within the genera of any of the above species.

Overexpression may be obtained using a variety of techniques familiar to those skilled in the art. For example, overexpression may be obtained by operably linking a gene encoding a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, or a gene product comprising a homologous polypeptide to a promoter which transcribes a higher level of mRNA encoding or comprising the gene product than does a wild type cell.

A variety of promoters may be used to overexpress the gene product described herein, including a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide. The promoters used to overexpress the gene product may be relatively strong promoters, promoters which possess a moderate level of activity, or relatively weak promoters and may be either constitutive or regulatable promoters. In some embodiments, several strains, each of which overexpresses the gene product to a different extent, may be used in order to optimize the degree of overexpression of the gene product.

In some embodiments, each of the gene products required for proliferation may be placed under the control of several different promoters of varying strengths to create several different strains which express the gene product at varying levels. The level of expression of the gene product in each of the strains is compared to that in wild type cells in order to identify a promoter which provides a desired level of expression relative to wild type cells (i.e. a desired level of overexpression or underexpression). The strain having the desired level of expression is then included in a culture or collection of strains to be contacted with a test compound as discussed below. Examples of suites of regulatable promoters having varying strengths that are useful for the expression of gene products at varying levels are described in U.S. Patent Application Serial Number 10/032,393, filed on December 21, 2002.

The promoter is selected to be active in the type of cell in which the gene product is to be expressed. For example, for overexpression of the gene product in mammalian cells, the gene encoding the gene product may be operably linked to promoters such as the SV40 promoter, the metallothionine promoter, the MMTV promoter, the RSV promoter, the tetP promoter, the adenovirus major late promoter or other promoters known to those skilled in the art. In yeast, the gene encoding the gene product may be operably linked to promoters such as the CYC1, ADHI,

ADHII, GAL1, GAL10, PHO5, PGK or other promoters used in the art. Similarly, in bacteria, the gene encoding the gene product may be operably linked to the , SP6, T3, trc promoter, lac promoter, temperature regulated lambda promoters, the *Bacillus* aprE and nprE promoters (U.S. Patent No. 5,387,521), the bacteriophage lambda P_L and P_R promoters (Renaut, et al., (1981) Gene 15: 81) the trp promoter (Russell, et al., (1982) Gene 20: 23), the tac promoter (de Boer et al., (1983) Proc. Natl. Acad. Sci. USA 80: 21), *B. subtilis* alkaline protease promoter (Stahl et al, (1984) J. Bacteriol. 158, 411-418) alpha amylase promoter of *B. subtilis* (Yang et al., (1983) Nucleic Acids Res. 11, 237-249) or *B. amyloliquefaciens* (Tarkinen, et al, (1983) J. Biol. Chem. 258, 1007-1013), the neutral protease promoter from *B. subtilis* (Yang et al, (1984) J. Bacteriol. 160, 15-21), T7 RNA polymerase promoter (Studier and Moffatt (1986) J Mol Biol. 189(1):113-30), *B. subtilis xyl* promoter or mutant tetR promoter active in bacilli (Geissendorfer & Hillen (1990) Appl. Microbiol. Biotechnol. 33:657-663), Staphylococcal enterotoxin D promoter (Zhang and Stewart (2000) J. Bacteriol. 182(8):2321-5), cap8 operon promoter from *Staphylococcus aureus* (Ouyang et al., (1999) J. Bacteriol. 181(8):2492-500), the lactococcal nisA promoter (Eichenbaum (1998) Appl Environ Microbiol. 64(8):2763-9), promoters from in *Acholeplasma laidlawii* (Jarhede et al., (1995) Microbiology 141 (Pt 9):2071-9), porA promoter of *Neisseria meningitidis* (Sawaya et al., (1999) Gene 233:49-57), the fbpA promoter of *Neisseria gonorrhoeae* (Forng et al., (1997) J. Bacteriol. 179:3047-3052), *Corynebacterium diphtheriae* toxin gene promoter (Schmitt and Holmes (1994) J. Bacteriol. 176(4):1141-9), the hasA operon promoter from Group A Streptococci (Alberti et al., (1998) Mol Microbiol 28(2):343-53), the rpoS promoter of *Pseudomonas putida* (Kojic and Venturi (2001) J. Bacteriol. 183:3712-3720), the *Acinetobacter baumannii* phosphate regulated *ppk* gene promoter (Gavigan et al., Microbiology 145:2931-7 (1999)); the *Acinetobacter baumannii adhC1* promoter which is induced under iron limitation and repressed when the cells are cultured in the presence of free inorganic iron (Echenique et al., Microbiology 147:2805-15 (2001)); the *flaB* promoter of pGK12 active in *Borrelia burgdorferi* (Sartakova et al., Proc Natl Acad Sci U S A. 97(9):4850-5 (2000)); the use of Ptrc promoter results in strong inducer-dependent expression in *Burkholderia spp* (Santos et al., FEMS Microbiol Lett 195(1):91-6 (2001)); the iron regulated *sodA* promoter of *Bordetella pertussis* (Graeff-Wohlleben et al., J Bacteriol 179(7):2194-201 (1997)); UV-inducible bcn and uviAB promoters in *Clostrdia spp* (Garnier and Cole Mol Microbiol 2(5):607-14 (1988)); the heat-inducible *clpB* promoter of *Campylobacter jejuni* (Thies et al., Gene 230(1):61-7 (1999)); promoters carrying bacteriophage C1 operator sites in *Klebsiella pneumoniae* (Schoefield et al, J Bacteriol 183(23):6947-50 (2001)); the *Proteus mirabilis ureR* promoter (Poore et al., J Bacteriol 183(15):4526-35 (2001)); and the heat-inducible *groESL* promoter in *Listeria monocytogenes*, and the IPTG inducible promoter in pLEX5BA (Krause et al., J. Mol. Biol. 274: 365 (1997). In another embodiment, which may be useful in *Staphylococcus aureus*, the promoter is a novel inducible promoter system, XylT5, comprising a modified T5 promoter fused to the *xylO* operator from the *xylA* promoter of *Staphylococcus aureus*. This promoter is described in U.S. Patent Application Serial Number 10/032,393. In another embodiment the promoter may be a two-

component inducible promoter system in which the T7 RNA polymerase gene is integrated on the chromosome and is regulated by *lac*UV5/ *lac*O (Brunschwig, E. and Darzins, A. 1992. Gene 111:35-41, and a T7 gene 10 promoter, which is transcribed by T7 RNA polymerase, is fused with a *lac*O operator. In another embodiment the promoter may be the promoters from the plasmids pEPEF3 or pEPEF14, which harbor xylose inducible promoters functional in *E. faecalis*, described in U.S. Patent Application Serial No. 10/032,393. Other promoters which may be used are familiar to those skilled in the art. In fungi, the gene encoding the gene product may be operably linked to the CaACT1 promoter (Morschhauser, Mol. Gen. Genet. 257: 412-420 (1998), or other promoters familiar to those skilled in the art. It will appreciated that other combinations of organisms and promoters may also be used in the present invention.

In some embodiments, overexpression may be achieved by using homologous recombination to replace the natural promoter which drives expression of the proliferation-required genes described herein with a regulatable promoter. For example, the methods described in U.S. Patent Application 09/948,993 may be used to place the gene required for proliferation under the control of a regulatable promoter. Examples of gene products, which are encoded by genes that can be overexpressed by regulatable promoters introduced by such promoter replacement methods include a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

Briefly, in some embodiments of these methods in which natural promoters are replaced by regulatable promoters, the cells may be haploid, such as bacterial cells. Regulatable promoters that are useful for promoter replacement in bacterial cells include, but are not limited to, the promoters described in U.S. Patent Application Serial Number 10/032,393 filed December 21, 2001. A linear promoter replacement cassette comprising a regulatable promoter flanked by nucleotide sequences having homology to the natural promoter is introduced into the cell. In some embodiments, the cassette also comprises a nucleotide sequence encoding a selectable marker or a marker whose expression is readily identified. The cassette may be a double stranded nucleic acid or a single stranded nucleic acid as described in U.S. Patent Application Serial Number 09/948,993. Upon homologous recombination, the natural promoter is replaced with the regulatable promoter, leaving the gene required for proliferation under the control of the regulatable promoter. Strains in which the gene required for proliferation is under control of the regulatable promoter are grown under conditions in which the regulatable promoter provides a level of the proliferation-required gene product which is above the level in a wild type cell. For example, the strains may be grown in the

presence of an inducer which induces expression from the regulatable promoter, or under conditions in which the action of a repressor on the regulatable promoter is reduced or eliminated.

Alternatively, rather than replacing the native promoters of each of the genes encoding a proliferation-required gene product described herein with a single desired replacement promoter, a plurality of replacement promoters which provide desired expression levels for the gene products to be overexpressed or underexpressed are used. The method is performed as described above except that rather than using a single labeled primer complementary to a nucleotide sequence within the single replacement promoter, a plurality of labeled primers complementary to suitable nucleotide sequences in the plurality of replacement promoters are used.

Alternatively, in embodiments in which the level or activity of proliferation-required gene products described herein is reduced by transcribing an antisense nucleic acid complementary to at least a portion of the genes encoding such gene products, the strains may be designed such that the length of the nucleotide sequence encoding the antisense nucleic acid is different for each gene. Amplification reactions are performed as described above using primers at each end of the gene encoding the antisense nucleic acid such that the amplification product corresponding to each gene has a unique length or a dye which allows it to be distinguished from other amplification products of the same length. Alternatively, the lengths of the nucleotide sequences encoding the antisense nucleic acids may not be unique for each gene, but the primers used in the amplification reaction may be selected such that the length of the amplification product corresponding to each gene is unique.

In another embodiment, the native promoters may be replaced with promoters which include therein or adjacent thereto a unique nucleotide sequence which is distinct from that present in the other replacement promoters in the strains in the culture or collection of strains. In this embodiment, each promoter includes or has adjacent thereto a unique "tag" which may be used to identify strains which proliferate more rapidly or more slowly in the culture or collection of strains. The tag may be detected using hybridization based methods or amplification based methods, including the amplification method which generates amplification products having a unique size for each proliferation required gene described above.

Alternatively, the native promoter which directs the transcription of the proliferation-required genes described herein may rendered regulatable by inserting a regulatory element into the chromosome of the cell via homologous recombination such that the regulatory element regulates the level of transcription from the promoter. Examples of gene products, which are encoded by genes that have promoters which can be rendered regulatable by regulatory elements inserted by such methods include a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino aeid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or

level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

A variety of regulatory elements may be used to regulate the expression of essential gene products described herein. The regulatory element may be an operator which is recognized by a repressor (e.g. lac, tet, araBAD repressors) or a nucleotide sequence which is recognized by a transcriptional activator. In some embodiments, the regulatory element may be a transcriptional terminator, a nucleotide sequence which introduces a bend in the DNA or an upstream activating sequence. A linear regulatory element insertion cassette comprising a regulatory element flanked by nucleotide sequences having homology to the natural promoter is introduced into the cell. In some embodiments, the cassette also comprises a nucleotide sequence encoding a selectable marker or a marker whose expression is readily identified. The cassette may be a double stranded nucleic acid or a single stranded nucleic acid as described in U.S. Patent Application Serial Number 09/948,993. Upon homologous recombination, the regulatory element is inserted into the chromosome, leaving the gene required for proliferation under the control of the regulatory element. Strains in which the gene required for proliferation is under control of the regulatory element are grown under conditions in which the regulatable promoter provides a level of the proliferation-required gene product which is above the level in a wild type cell. For example, the strains may be grown in the presence of an inducer which induces expression from the promoter, or under conditions in which the action of a repressor on the promoter is reduced or eliminated. It will be appreciated that the amplification method which generates amplification products having a unique size for each proliferation required gene may be used to detect strains which are overrepresented or underrepresented in the culture or collection of strains. For example, if desired, primers complementary to a nucleotide sequence within the regulatory element may be used in the amplification reaction.

The promoter replacement cassette or regulatory element insertion cassette may be a double stranded nucleic acid, such as an amplicon generated through PCR or other amplification methods, or a single stranded nucleic acid, such as an oligonucleotide. For example, single stranded nucleic acids may be introduced into the chromosome using the methods described in Ellis et al., PNAS 98: 6742-6746, 2001.

In some embodiments, the cell into which the promoter replacement cassette or regulatory element insertion cassette is introduced has an enhanced frequency of recombination. For example, the cells may lack or have a reduced level or activity of one or more exonucleases which would ordinarily degrade the DNA to be inserted into the chromosome. In further embodiments, the cells may both lack or have reduced levels of exonucleases and express or overexpress proteins involved in mediating homologous recombination. For example, if the methods are performed in *Escherichia coli* or other enteric prokaryotes, cells in which the activity of exonuclease V of the RecBCD recombination pathway, which degrades linear nucleic acids, has been reduced or eliminated, such as recB, recC, or recD mutants may be used. In some embodiments, the cells have

mutations in more than one of the recB, recC, and recD genes which enhance the frequency of homologous recombination. For example the cells may have mutations in both the recB and recC genes.

The promoter replacement or regulatory element insertion methods may also be performed in *Escherichia coli* cells in which the activity of the RecET recombinase system of the Rac prophage has been activated, such as cells which carry an sbcA mutation. The RecE gene of the rac prophage encodes ExoVIII a 5'-3' exonuclease, while the RecT gene of the Rac prophage encodes a single stranded DNA binding protein which facilitates renaturation and D-loop formation. Thus, the gene products of the RecE and RecT genes or proteins with analogous functions facilitate homologous recombination. The RecE and RecT genes lie in the same operon but are normally not expressed. However, sbcA mutants activate the expression the RecE and RecT genes. In some embodiments, the methods may be performed in cells which carry mutations in the recB and recC genes as well as the sbcA mutation. The RecE and RecT gene may be constitutively or conditionally expressed. For example, the methods may be performed in *E. coli* strain JC8679, which carries the sbcA23, recB21 and recC22 mutations.

In some embodiments, the methods may be performed in *Escherichia coli* cells in which recombination via the RecF pathway has been enhanced, such as cells which carry an sbcB mutation.

It will be appreciated that the RecE and RecT gene products, or proteins with analogous functions may be conditionally or constitutively expressed in prokaryotic organisms other than *E. coli*. In some embodiments, these proteins may be conditionally or constitutively expressed in *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,*

Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. For example, plasmids encoding these gene products may be introduced into the organism. If desired, the coding sequences encoding these gene products may be optimized to reflect the codon preferences of the organism in which they are to be expressed. Similarly, in some embodiments, the organism may contain mutations analogous to the recB, recC, recD, sbcA or sbcB mutations which enhance the frequency of homologous recombination.

In further embodiments, the promoter replacement or regulatory element insertion methods may be conducted in cells which utilize the Red system of bacteriophage lambda (λ) or analogous systems from other phages to enhance the frequency of homologous recombination. The Red system contains three genes, (γ, β and *exo* whose products are the Gam, Bet and Exo proteins (see Ellis et al. PNAS 98:6742-6746, 2001. The Gam protein inhibits the RecBCD exonuclease V, thus permitting Beta and Exo to gain access to the ends of the DNA to be integrated and facilitating homologous recombination. The Beta protein is a single stranded DNA binding protein that promotes the annealing of a single stranded nucleic acid to a complementary single stranded nucleic acid and mediates strand exchange. The Exo protein is a double-stranded DNA dependent 5'-3' exonuclease that leaves 3' overhangs that can act as substrates for recombination. Thus, constitutive or conditional expression of the λ Red proteins or proteins having analogous functions facilitates homologous recombination.

It will be appreciated that the λ Beta, Gam and Exo proteins, or proteins with analagous functions may be expressed constitutively or conditionally in prokaryotic organisms other than *E. coli*. In some embodiments, these proteins may be conditionally or constitutively expressed in *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,*

Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. For example, plasmids encoding these gene products may be introduced into the organism. If desired, the coding sequences encoding these gene products may be optimized to reflect the codon preferences of the organism in which they are to be expressed.

In some embodiments, the cells may have an increased frequency of homologous recombination as a result of more than one of the aforementioned characteristics. In some embodiments, the enhanced frequency of recombination may be a conditional characteristic of the cells which depends on the culture conditions in which the cells are grown. For example, in some embodiments, expression of the λ Red Gam, Exo, and Beta proteins or recE and recT proteins may be regulated. Thus, the cells may have an increased frequency of homologous recombination as a result of any combination of the aforementioned characteristics. For example, in some embodiments, the cell may carry the sbcA and recBC mutations.

In some embodiments, a linear double stranded DNA to be inserted into the chromosome of the organism is introduced into an organism constitutively or conditionally expressing the recE and recT or the λ Beta, Gam and Exo proteins or proteins with analogous functions as described above. In some embodiments, the organism may be *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,*

Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments, the double stranded DNA may be introduced into an organism having the recBC and sbcA mutations or analogous mutations.

In other embodiments, a single stranded DNA to be inserted into the chromosome of the organism is introduced into an organism expressing the λ Beta protein or a protein with an analogous function. In some embodiments the single stranded DNA is introduced into an organism expressing both the λ Beta and Gam proteins or proteins with analogous functions. In further embodiments, the single stranded DNA is introduced into an organism expressing the λ Beta, Gam and Exo proteins or proteins with analogous functions. The λ proteins or analogous proteins may be expressed constitutively or conditionally. In some embodiments, the organism may be *Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata* (also called *Torulopsis glabrata*), *Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr* (also called *Candida pseudotropicalis*), *Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis* or any species falling within the genera of any of the above species.

In some embodiments, the linear nucleic acid may be introduced into the chromosome of a first organism which has an enhanced frequency of homologous recombination and then transferred to a second organism which is less amenable to direct application of the present methods. For example, the linear nucleic acid may be introduced into the chromosome of *E. coli* and transferred

into a second organism via conjugation or transduction. After introduction into the second organism, the nucleic acid is inserted into the chromosome of the second organism via homologous recombination, thereby effectively transferring the regulatory element from the chromosome of the first organism into the corresponding location in the chromosome of the second organism.

In other embodiments, the cells may be diploid cells, such as fungal cells. In some embodiments, one copy of the gene encoding the proliferation-required gene product may be disrupted, rendering it inactive. In further embodiments, one copy of the gene encoding the proliferation-required gene product may be disrupted and the other copy of the gene encoding the proliferation-required gene product may be placed under the control of a regulatable promoter. Such strains may be generated by disrupting the first copy of the gene encoding the proliferation-required gene product by homologous recombination using a disruption cassette comprising a nucleotide sequence encoding an expressible dominant selectable marker flanked on each side by nucleic acids homologous to the target sequence to be disrupted. The second copy of the gene encoding the proliferation-required gene product may be placed under the control of a regulatable promoter by homologous recombination using a promoter replacement cassette comprising a regulatable promoter flanked on each side by nucleic acids homologous to the natural promoter for the proliferation-required gene. The promoter replacement cassette may also include a nucleotide sequence encoding a selectable marker located 5' of the regulatable promoter but between the nucleic acids homologous to the natural promoter.

In other embodiments, overexpression may be achieved by operably linking a proliferation-required gene product described herein to a desired promoter in a vector. The vector may be a vector which replicates extrachromosomally or a vector which integrates into the chromosome. For example, if the vector is to be used in bacterial cells, the vector may be a pBR322 based vector or a bacteriophage based vector such as P1 or lambda. If the vector is to be used in *Saccharomyces cerevisae*, it may be a vector based on the 2 micron circle or a vector incorporating a yeast chromosomal origin of replication. If the vector is to be used in mammalian cells, it may be a retroviral vector, SV40 based vector, a vector based on bovine papilloma virus, a vector based on adenovirus, or a vector based on adeno-associated virus. If the vector is to be used in *Candida albicans* it may be a vector comprising a promoter selected from the group consisting of the CaPCK1, MET25, MAL2, PHO5, GAL1,10, STE2 or STE3 promoters. In some embodiments, the vectors described in the following publications may be used: CIp10, an efficient and convenient integrating vector for *Candida albicans*. Murad et al., Yeast 16(4):325-7 (2000); Transforming vector pCPW7, Kvaal et al., : Infect Immun 67(12):6652-62 (1999); Transforming vector pCWOP16, Kvaal et al., : Infect Immun 65(11):4668-75 (1997); double-ARS vector, pRM1, to be used for direct cloning in Ca by complementation of the histidine auxotrophy of strain CA9, Pla et al., Gene 165(1):115-20 (1995); pMK16, that was developed for the transformation of *C. albicans* and carries an ADE2 gene marker and a *Candida* autonomously replicating sequence (CARS) element promoting autonomous replication (cited in Sanglard and Fiechter Yeast 8(12):1065-75

(1992); A plasmid vector (denoted pRC2312) was constructed, which replicates autonomously in *Escherichia coli, Saccharomyces cerevisiae* and *Candida albicans*. It contains LEU2, URA3 and an autonomously replicating sequence (ARS) from *C. albicans*, Cannon et al., Mol Gen Genet 235(2-3):453-7 (1992); Expression vector (CIp10-MAL2p) for use in *Candida albicans* has been constructed in which a gene of interest can be placed under the control of the CaMAL2 maltase promoter and stably integrated at the CaRP10 locus (Backen et al., Yeast 16(12):1121-9 (2000)); (Volker, R. S., A. Sonneborn, C. E. Leuker, and J. F. Ernst. 1997. Efg1p, an essential regulator of morphogenesis of the human pathogen *Candida albicans*, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. EMBO **16**:1982-1991.); and a *C. albicans* transformation vector containing the *C. albicans* URA3 gene, a Candida ARS sequence, and a portion of the *Saccharomyces cerevisiae* 2 microns circle containing the replication origin was constructed. Goshorn et al., Infect Immun 60(3):876-84 (1992). A variety of other vectors suitable for use in foregoing organisms or in any other organism in which the present invention is to be practiced are familiar to those skilled in the art.

Underexpression of a proliferation-required gene product described herein may be obtained in a variety of ways. For example, in one embodiment underexpression of the proliferation-required gene product may be achieved by providing an agent, such as an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, an antisense nucleic acid comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a nucleic acid complementary to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a nucleic acid complementary to a nucleic acid which encodes at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of a polypeptide sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, an antisense nucleic acid comprising at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a homologous nucleic acid, a nucleic acid complementary to a homologous coding nucleic acid, a nucleic acid complementary to at least 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, 150, 200, 300, 400, or 500 consecutive nucleotides of a homologous coding nucleic acid, a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide, or a nucleic acid complementary to a nucleic acid which encodes at least 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids of a homologous polypeptide, which reduces the level or activity of the gene product within the cell. In one embodiment, the agent may comprise an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ

ID NOs.: 1-6213 which is complementary to a nucleic acid encoding the proliferation-required gene product or complementary to a portion of a nucleic acid encoding the proliferation-required gene product.

In one example of antisense-inhibition-based underexpression, a nucleic acid which encodes the antisense nucleic acid may be operably linked to a regulatable promoter. When grown under appropriate conditions, such as media containing an inducer of transcription or an agent which alleviates repression of transcription, the antisense nucleic acid is expressed in the cell, thereby reducing the level or activity of the gene product within the cell. In some embodiments, the concentration of the inducer of transcription or the agent which alleviates repression of transcription may be varied to provide optimal results. Such methods have been described previously herein and in U.S. Patent Application Serial Number 09/815,242, U.S. Patent Application Serial Number 09/492,709, U.S. Patent Application Serial Number 09/711,164, or U.S. Patent Application Serial Number 09/741,669.

Alternatively, underexpression of a proliferation-required gene product described herein may be achieved by constructing strains in which the expression of the gene product is under the control of a constitutive or regulatable promoter using methods such as those described above with respect to methods in which the gene product is overexpressed. To provide cells which underexpress the gene product, the cells are grown under conditions in which the gene product is expressed at a level lower than that of a wild type cell. For example, the cells may be grown under conditions in which a repressor reduces the level of transcription from the regulatable promoter.

In other embodiments, underexpression may be achieved by operably linking the gene required for proliferation to a desired promoter in a vector as described above with respect to embodiments in which gene products required for proliferation are overexpressed. In some embodiments, the vector may be present in cells in which the chromosomal copy or copies of the gene has been disrupted.

Examples of gene products, which are encoded by genes that can be underexpressed using methods such as those described above with respect to methods in which the gene product is overexpressed include a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

One embodiment of the invention includes a method for identifying a gene product described herein on which a compound which inhibits the proliferation of an organism acts. The method employs a culture which comprises a mixture of strains of the organism. At least some of the strains in the culture overexpress a different gene product which is required for the proliferation

of the organism. Preferably, each of the strains in the culture overexpresses a different gene product which is required for proliferation of the organism (i.e. all of the strains in the culture overexpress a gene product which is required for proliferation of the organism). For example, the gene product which is overexpressed in each strain may be a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

Strains that overexpress the proliferation-required gene product may be obtained using the methods described above. The culture may comprise any number of strains which overexpress a gene product required for proliferation. For example the culture may comprise at least two strains, at least 10 strains, at least 20 strains, at least 30, strains, at least 50 strains, at least 100 strains, at least 300 strains or more than 300 strains which overexpress a gene product required for proliferation. In some embodiments, the culture may comprise strains which in aggregate overexpress all or most of the gene products required for proliferation of the organism.

The culture is contacted with a compound which inhibits proliferation of the organism. The compound may be a candidate drug compound obtained from any source. For example, the compound may be a compound generated using combinatorial chemistry, a compound from a natural product library, or an impure or partially purified compound, such as a compound in a partially purified natural extract. The culture is contacted with a sufficient concentration of the compound to inhibit the proliferation of strains of the organism in the culture which do not overexpress the gene product on which the compound acts, such that strains which overexpress said gene product on which the compound acts proliferate more rapidly in the culture than strains which do not overexpress said gene product on which said compound acts. Thus, after a sufficient period of time, the strain which overexpresses the gene product on which the compound acts will be more prevalent in the culture than strains which do not overexpress the gene product on which the compound acts. In a preferred embodiment, the growth conditions and incubation period are selected so that only one strain, the strain overexpressing the target of the compound, is recovered from the culture. Thus, in one embodiment, a plurality of cultures containing a plurality of strains each of which overexpresses a different proliferation-required gene product may be grown in the presence of varying concentrations of the compound. In addition to varying the compound concentrations, in embodiments where expression of the proliferation-required gene product is under the control of a regulatable promoter, the plurality of cultures may be grown at varying concentrations of an agent which regulates the level of expression from the promoter, such as an inducer or an agent which reduces the effect of a repressor on transcription from the promoter. It

will be appreciated, that the cultures may be grown in liquid medium in the presence of the compound whose target is to be identified (and where appropriate in the presence of an agent which regulates the level of expression from the promoter) or alternatively, a liquid culture comprising the strains which overexpress the proliferation-required gene products may be grown in the absence of the compound whose target is to be identified and then introduced onto a solid medium containing the compound (and, where appropriate, also containing an agent which regulates the level of expression from the promoter).

The identity of the overexpressed gene product which is the target of the compound may be determined using a variety of methods. For example, in some embodiments of the present invention, the nucleic acids present in the culture or collection of strains which was contacted with the compound may be compared to the nucleic acids present in a control culture or collection of strains which was not contacted with the compound to identify nucleic acids which are overrepresented in the culture or collection of strains contacted with the test compound relative to the control culture or collection of strains. Alternatively, in some embodiments, the nucleic acids present in a culture or collection of strains contacted with the test compound may be analyzed to identify those nucleic acids which are present without comparison to a control culture or collection of strains.

In some embodiments, the strains which proliferated more rapidly in the culture or collection of strains, i.e. strains having an enhanced ability to proliferate in the presence of a test compound relative to other strains in the culture or collection of strains, are identified as follows. Amplification products which are correlated with each of the overexpressed genes and which are distinguishable from one another are obtained from a culture or collection grown in the presence of a test compound. The amplification products are distinguished from one another to determine whether a particular amplification product is overrepresented in the culture or collection of strains. In some embodiments, the amplification products corresponding to each of the gene products have lengths which permit them to be distinguished from one another. In another embodiment, one or more of the amplification products have similar or identical lengths but are distinguishable from one another based on a detectable agent, such as a dye, attached thereto. In some embodiments, amplification products which are overrepresented are identified by comparing the amplification products from the culture or collection of strains which was contacted with the test compound to the amplification products from a culture or collection of strains which was not contacted with the test compound. Alternatively, amplification products which are overrepresented may be identified by simply identifying the amplification products obtained from the culture or collection of strains contacted with the test compound (for example, only one or a few strains may have proliferated in the presence of the test compound). The above methods for generating distinguishable amplification products may be used in conjunction with any of the methods for generating strains which overexpress gene products required for proliferation described herein in order to facilitate the

identification of strains which proliferate more rapidly or more slowly in the presence of a test compound.

For example, in some embodiments of the present invention, each of the native promoters of each of the genes encoding gene product required for proliferation are replaced by a single desired replacement promoter. After growth of the culture or collection of strains containing the strains in which the promoters have been replaced in the presence of a test compound for a desired period of time, an amplification reaction is performed on nucleic acids obtained from the culture as follows.

The nucleic acids from the culture or collection of strains may be divided into at least two aliquots if desired. In a preferred embodiment the nucleic acids from the culture or collection of strains are divided into four aliquots. A single primer complementary to a nucleotide sequence within the replacement promoter , within the proliferation required genes, or within nucleic acid sequences adjacent to the promoter or proliferation required genes is divided into at least two portions, one portion for each aliquot of nucleic acids. Each portion of the primer is labeled with a distinct detectable dye, such as the 6FAM™, TET™, VIC™, HEX™, NED™, and PET™ dyes obtainable from Applied Biosystems (Foster City, CA). For example, the DS-31 or DS-33 dye sets available from Applied Biosystems (Foster City, CA) may be used to label the primers. Alternatively, the HEX™, NED, JOE, TMR and TET™ dyes available from Amersham Biosciences may be used. Thus, if the nucleic acids from the culture are not divided into aliquots, a single primer labeled with a single dye may be used. If the nucleic acids from the culture are divided into aliquots, at least 2, at least 3, at least 4 or more than 4 primers labeled with distinguishable dyes may be used. Each of the portions of labeled primers are added to each of the aliquots of the nucleic acids from the culture or collection of strains such that each aliquot of nucleic acid receives a single labeled primer with a single detectable dye thereon. In some embodiments, the primers are divided into 3 portions, 4 portions or more than 4 portions, with each portion having a dye which is distinguishable from the dyes on the other portions thereon.

Each of the aliquots of nucleic acids also receives a set of unlabeled primers, with each of the unlabeled primers being complementary to a nucleotide sequence within the promoter, within a nucleotide sequence which is unique to one of the genes encoding gene products required for proliferation which were placed under the control of the replacement promoter, or within nucleotide sequences adjacent to the promoter or proliferation required genes. Each of the aliquots receives primers unique to 1/N proliferation required genes which were placed under the control of the replacement promoter, where N is the number of aliquots (i.e. if the culture or collection of strains consisted of 100 strains in which a gene required for proliferation was placed under the control of the replacement promoter and was divided into four aliquots, then each of the four aliquots of nucleic acids from the culture or collection of strains would receive primers complementary to 25 of the genes). The unlabeled primers are selected so that each will yield an amplification product having a length distinguishable from the length of the amplification product produced with the other

unlabeled primers. Preferably, the amplification products are between about 100-about 400 nucleotides in length, but any lengths which may be distinguished from each other may be used. In addition, in some of the embodiments some of the amplification products may have identical or very similar lengths but be distinguishable from one another due to labeling with distinguishable dyes.

A nucleic acid amplification reaction is conducted on each of the nucleic acid aliquots. The amplification products are then separated by length to identify amplification products having increased representation in the culture or collection of strains (i.e. amplification products derived from cells which proliferated more rapidly in the culture or collection of strains). The amplification products are then correlated with the corresponding genes to determine which strains proliferated more rapidly in the culture or collection of strains. If desired, amplification products having increased representation in the culture may be identified by comparing the amplification products obtained from a culture or collection of strains which was contacted with the compound to amplification products obtained from a control culture or collection of strains which was not contacted with the compound. Alternatively, if desired, the amplification products which are obtained from a culture which was contacted with the compound may be directly identified without comparison to a control culture which was not contacted with the compound.

For example, in some embodiments, the amplification products from each of the nucleic acid aliquots are pooled and subjected to capillary electrophoresis. The amplification products are detected by detecting the fluorescent dyes attached thereto and their lengths are determined to identify those amplification products having increased or decreased representation in the culture or collection of strains. Figures 2A and 2B illustrate one embodiment of this method in which the absence of an amplification product from an amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation indicates that a test compound acts on the gene corresponding to the missing amplification product. It will be appreciated that the method may also be used to identify an amplification product which is overrepresented in an amplification reaction conducted on a culture or collection of strains overexpressing genes required for proliferation because the test compound acted on the corresponding gene.

Alternatively, in another embodiment, a first amplification reaction is performed on nucleic acids obtained from a culture or collection of strains which was contacted with the compound using a first primer complementary to a nucleotide sequence present upstream or downstream of all of the overexpressed genes (such as a primer complementary to a nucleotide sequence in a replacement promoter upstream of all of the overexpressed genes) and a set of primers complementary to a nucleotide sequence unique to each of the strains (such as a primer complementary to a nucleotide sequence within each of the proliferation-required genes). One of the two amplification primers for each of the proliferation required genes is labeled with a dye as described above. Preferably, the common primer complementary to a nucleotide sequence upstream or downstream of all of the

overexpressed genes is labeled with the dye. The primers used in the amplification reaction are designed so that the amplification product corresponding to each proliferation-required gene has a unique length or a dye which allows it to be distinguished from other amplification products of the same length. A second amplification reaction is conducted on a control culture or collection of strains which was not contacted with the compound using the same primers as in the first amplification reaction. The amplification products from the first amplification reaction are compared to those from the second amplification reaction to identify one or more amplification products which are overrepresented in the culture or collection of strains. For example, the amplification products from the first amplification reaction may be run in a separate lane of a polyacrylamide gel or a separate capillary than the amplification products from the second amplification reaction and the two lanes or capillaries are compared to one another. If desired, in the embodiment where the amplification products from the first amplification reaction are run in a different lane or capillary than the amplification products from the second amplification reaction, the same dye may be used to label the primers in the first and second amplification reactions. Alternatively, if desired, different dyes may be used to label the primers in the first and second amplification reactions. If desired, in the embodiment where the amplification products from the first amplification reaction are run in a different lane or capillary than the amplification products from the second amplification reaction, the same dye may be used to label the primers in the first and second amplification reactions. Alternatively, if desired, different dyes may be used to label the primers in the first and second amplification reactions.

Alternatively, in some embodiments, the primers in the second amplification reaction are labeled with a different dye which is distinguishable from the dye used in the first amplification reaction. In this embodiment, the amplification reactions may be pooled and run in the same lane on a polyacrylamide gel or in the same capillary and the products from each amplification reaction are compared by comparing the amount of each dye present for each amplification product. Figures 3A and 3B illustrate one embodiment of this method in which the absence of an amplification product from the amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation which was contacted with the compound indicates that a test compound acts on the gene corresponding to the missing amplification product. It will be appreciated that the method may also be used to identify an amplification product which is overrepresented in an amplification reaction conducted on a culture or collection of strains overexpressing genes required for proliferation because the test compound acted on the corresponding gene.

If desired, rather than dividing the culture into aliquots, individual amplification reactions may be conducted on nucleic acids obtained from the culture or collection of strains. Each amplification reaction contains primers which will yield an amplification product specific for only one of the proliferation required genes. The resulting amplification products from each of the

individual amplification reactions are pooled and amplification products having increased representation in the culture are identified as described above.

In another embodiment, a culture or collection of strains in which gene products required for proliferation are overexpressed from regulatable promoters which replaced the native promoters of the genes encoding these gene products is allowed to grow in the presence of a test compound for a desired number of generations. Preferably, the culture or collection of strains is allowed to grow in the presence of the test compound for at least 20 generations. Nucleic acids are isolated from the culture or collection of strains and an amplification reaction is performed using a primer which is complementary to a nucleotide sequence within the replacement promoter(s) or a nucleotide sequence adjacent to the a 5' end thereof and primers which are complementary to a nucleotide sequence within the proliferation required genes or nucleotide sequences adjacent thereto. The resulting amplification product(s) is directly sequenced using a primer complementary to a nucleotide sequence within the replacement promoter.

In one embodiment of the present invention, the vector containing the nucleotide sequence encoding the proliferation-required gene product is obtained from a strain which proliferated more rapidly in the culture using methods such as plasmid preparation techniques. Nucleic acid sequencing techniques are then employed to determine the nucleotide sequence of the gene which was overexpressed.

Alternatively, the identity of the overexpressed gene product which is the target of the compound may be determined by performing a nucleic acid amplification reaction, such as a polymerase chain reaction (PCR), to identify the nucleotide sequence of the gene which was overexpressed. For example, aliquots of a nucleic acid preparation, such as a purified plasmid, from the strain which is recovered from the culture may each be contacted with pairs of PCR primers which would amplify a different proliferation-required gene to determine which pair of primers yields an amplification product.

An alternative method for determining the identity of the gene product described herein which is the target of the compound involves obtaining a nucleic acid array, such as a DNA chip, which contains each of the proliferation-required genes which were overexpressed in the strains in the culture. Each proliferation-required gene occupies a known location in the array. A nucleic acid preparation, such as a plasmid preparation, from the recovered strain is labeled with a detectable agent, such as radioactive or fluorescent moiety, and placed in contact with the nucleic acid array under conditions which permit the labeled nucleic acid to hybridize to complementary nucleic acids on the array. The location on the array to which the labeled nucleic acids hybridize is determined to identify the gene which was overexpressed in the recovered strain. If desired the hybridized nucleic acids from a culture which was contacted with the compound may be compared to the hybridized nucleic acids from a control culture which was not contacted with the compound. Alternatively, the hybridized nucleic acids from a culture which was contacted with the compound may be directly identified without comparison to nucleic acids from a control culture.

In some instances, more than one strain may proliferate more rapidly in the presence of the compound. This may result from a variety of causes. For example, the concentration of the compound may not have been high enough to restrict proliferation only to cells which overexpress one gene product (i.e. the target gene product). While strains which overexpress the target gene product will be the most prevalent strain in the culture, other strains may also have proliferated. In such instances, the identity of the gene product in the strain which is most prevalent in the culture may be identified by quantitating the levels of each of the genes encoding proliferation-required proteins in the culture. This may be accomplished by quantitative PCR, DNA sequencing, hybridization, or array technology as described above.

In other instances, multiple strains will exhibit more rapid proliferation in the culture as a result of a common functional attribute. For example, the strains which proliferate more rapidly may each overexpress a gene product with a common enzymatic activity, such as serine protease activity for example. Alternatively, the strains which proliferate more rapidly may each overexpress a gene product with a common functional domain, such as a cAMP binding domain. In such instances, the common attribute of the strains which proliferate more rapidly may provide information as to the mode of action of the compound or the biochemical activity of the target of the compound. For example, if all of the overexpressed genes in the strains which proliferated more rapidly are serine proteases, the compound acts by inhibiting serine protease activity and the target protein is a serine protease. If desired, the compound may be derivatized and the efficacy of the derivatized compound against each of the strains which proliferated more rapidly may be assessed as described herein in order to identify derivatives which are capable of interacting with a wide range of targets sharing a common activity or binding site (i.e. derivatives which have a greater ability to inhibit the proliferation of all the strains than the original compound) or to identify derivatives having greater specificity for a desired target (i.e. derivatives which have a greater specificity for one of the strains than the original compound). For example, it is possible that a nonessential gene product expressed in the cell might also bind to the initial test compound in addition to the gene product required for proliferation. In such an instance, it is desirable to obtain a derivative of the initial test compound which is specific for the gene product required for proliferation. In addition, it is possible that two gene products required for proliferation might bind to the initial test compound but specificity for one of the gene products is desired.

Rather than employing a single culture which contains multiple strains each of which overexpresses a proliferation-required gene product described herein, the methods of the present invention may be performed using an array of individual strains (i.e. a collection of strains) each of which overexpresses a different proliferation-required gene product. For example, individual strains each overexpressing a different proliferation-required gene product may be grown in different wells of a multiwell plate. Each well is contacted with the compound (and, where appropriate an agent which regulates the level of expression from the promoter). The level of proliferation of the strains in each of the wells is determined to identify a strain which proliferated

more rapidly. The identity of the overexpressed gene product in the strain that proliferated more rapidly is determined as described above.

In another embodiment, individual strains each overexpressing a different proliferation-required gene product (i.e. a collection of strains) are grown at different locations on a solid medium, such as an agar plate. The medium contains the compound and where appropriate an agent which regulates the level of expression from the promoter). The level of proliferation of each of the strains is determined to identify a strain which proliferated more rapidly. The identity of the overexpressed gene product in the strain that proliferated more rapidly is determined as described above.

The above methods may be used to prioritize compound development or to determine whether the compound has been previously identified or whether the target of the compound is the target of a previously identified drug. In particular, if the product is a natural product, it is advantageous to determine whether it has been previously identified prior to investing significant effort in developing it. Thus, in some embodiments of the present invention, the target of a partially purified or purified natural product or a compound produced by combinatorial chemistry is identified using the methods described above and compared to the targets of known drugs. If the target is identical to that of a known drug, further development of the compound is halted.

Alternatively, an array of strains each of which overexpresses a different gene product described herein (i.e. a collection of strains) is grown on solid medium containing a compound to be evaluated. The location of each strain in the array and the gene product overexpressed by that strain is known. The pattern of colonies which grow in the presence of the compound is evaluated and compared to the pattern of colonies which grow in the presence of previously identified drugs. If the pattern of colonies which grow in the presence of the compound being evaluated is the same as the pattern of colonies which grow in the presence of a previously identified drug, further development of the compound is halted.

Additionally in some embodiments, the sequence of the gene product in a strain which proliferated more rapidly in the assays described above is compared to the sequence of gene products from heterologous organisms to determine the likely spectrum of species whose growth would be inhibited by the compound. If the gene product has a high degree of homology to gene products from heterologous species, it is likely that the compound would also inhibit the growth of these heterologous species. Homology may be determined using any of a variety of methods familiar to those skilled in the art. For example, homology may be determined using a computer program such as BLASTP or FASTA. The ability of the compound to inhibit the growth of the heterologous species may then be confirmed by comparing the growth of cells of the heterologous species in the presence and absence of the compound.

Current methods for identifying the target of compounds which inhibit cellular proliferation are laborious and time consuming. The above methods may be employed to allow the targets of a large number of compounds to be rapidly identified. In such methods, the methods described above

are simultaneously performed for each of a large number of compounds. For example, the compounds may be members of a library of compounds generated using combinatorial chemistry or members of a natural product library. In such methods, a plurality of cultures each comprising a plurality of strains each of which overexpresses a different gene product required for proliferation or a plurality of collections of individual strains each of which overexpresses a different gene product required for proliferation is obtained. Each culture or collection of strains is contacted with a different compound in the library and the target of the compound is identified as described above.

In another embodiment, the gene product described herein on which a compound which inhibits the proliferation of an organism acts is identified using a culture which comprises a mixture of strains of the organism including strains which underexpress a different gene product which is required for proliferation of the organism (i.e. at least some of the strains in the culture underexpress a gene product which is required for proliferation of the organism). Preferably, each of the strains in the culture underexpress a different a gene product which is required for the proliferation of the organism (i.e. all of the strains in the culture underexpress a gene product which is required for the proliferation of the organism). In some embodiments, the culture comprises at least one strain which underexpresses a gene product selected from the group consisting of a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

Strains underexpressing the proliferation-required gene products described herein may be obtained using the methods described above. The culture may comprise any number of strains. For example the culture may comprise at least two strains, at least 10 strains, at least 20 strains, at least 30, strains, at least 50 strains, at least 100 strains, at least 300 strains or more than 300 strains which underexpress a gene product required for proliferation. In some embodiments, the strains in the culture in aggregate may underexpress all or most of the gene products required for proliferation of the organism.

The culture is contacted with a compound which inhibits proliferation of the organism. The compound may be a candidate drug compound obtained from any source. For example, the compound may be a compound generated using combinatorial chemistry, a compound from a natural product library, or an impure or partially purified compound, such as a compound in a partially purified natural extract. The culture is contacted with a sufficient concentration of the compound to inhibit the proliferation of strains of the organism in the culture which underexpress the gene product on which the compound acts, such that strains which do not underexpress the gene product on which the compound acts proliferate more rapidly in the culture than strains which do

underexpress said gene product on which said compound acts. Thus, after a sufficient period of time, the strain which underexpresses the gene product on which the compound acts will be less prevalent in the culture than strains which do not underexpress the gene product on which the compound acts. In one embodiment, the growth conditions and incubation period are selected so that only one strain, the strain underexpressing the target of the compound, proliferates at a reduced rate in the culture. In another embodiment, the growth conditions may be selected so that the strain underexpressing the target of the compound is not recovered from the culture. Thus, in one embodiment, a plurality of cultures containing a plurality of strains each of which underexpresses a different proliferation-required gene product may be grown in the presence of varying concentrations of the compound. In addition to varying the compound concentrations, in embodiments where expression of the proliferation-required gene product is under the control of a regulatable promoter, the plurality of cultures may be grown at varying concentrations of an agent which regulates the level of expression from the promoter, such as an inducer or an agent which reduces the effect of a repressor on transcription from the promoter. It will be appreciated, that the cultures may be grown in liquid medium in the presence of the compound whose target is to be identified (and where appropriate in the presence of an agent which regulates the level of expression from the promoter) or alternatively, a liquid culture comprising the strains which underexpress the proliferation-required gene products may be grown in the absence of the compound whose target is to be identified and then introduced onto a solid medium containing the compound (and, where appropriate, also containing an agent which regulates the level of expression from the promoter).

The identity of the underexpressed gene product which is the target of the compound may be determined using a variety of methods. For example, in some embodiments of the present invention, the nucleic acids present in the culture or collection of strains which was contacted with the compound may be compared to the nucleic acids present in a control culture or collection of strains which was not contacted with the compound to identify nucleic acids which are underrepresented in the culture or collection of strains contacted with the test compound relative to the control culture or strains. Alternatively, in some embodiments, the nucleic acids present in a culture or collection of strains contacted with the test compound may be analyzed to identify those nucleic acids which are missing or present at reduced levels without comparison to a control culture or collection of strains.

In some embodiments of the present invention, the strains which proliferated more slowly in the culture or collection of strains, i.e. strains having an decreased ability to proliferate in the presence of a test compound or which do not proliferate in the presence of a test compound, are identified as follows. Amplification products which are correlated with each of the underexpressed genes and which are distinguishable from one another are obtained from a culture or collection grown in the presence of a test compound. The amplification products are distinguished from one another to determine whether a particular amplification product is underrepresented in the culture or collection of strains. In some embodiments, the amplification products corresponding to each of the

gene products have lengths which permit them to be distinguished from one another. In another embodiment, one or more of the amplification products have similar or identical lengths but are distinguishable from one another based on a detectable agent, such as a dye, attached thereto. In some embodiments, amplification products which are underrepresented are identified by comparing the amplification products from the culture or collection of strains which was contacted with the test compound to the amplification products from a culture or collection of strains which was not contacted with the test compound. Alternatively, amplification products which are underrepresented in the culture or collection of strains may be identified simply by determining which amplification products are missing or present at reduced levels in the culture or collection of strains. The above methods for generating distinguishable amplification products may be used in conjunction with any of the methods for generating strains which underexpress gene products required for proliferation described herein in order to facilitate the identification of strains which proliferate more slowly in the presence of a test compound.

For example, in some embodiments of the present invention, each of the native promoters of each of the genes encoding gene product required for proliferation are replaced by a single desired replacement promoter. After growth of the culture or collection of strains containing the strains in which the promoters have been replaced in the presence of a test compound for a desired period of time, an amplification reaction is performed on nucleic acids obtained from the culture as follows.

The nucleic acids from the culture or collection of strains are divided into at least two aliquots. In a preferred embodiment the nucleic acids from the culture or collection of strains are divided into four aliquots. A single primer complementary to a nucleotide sequence within the replacement promoter , within the proliferation required genes, or within nucleic acid sequences adjacent to the promoter or proliferation required genes is divided into four groups Each group is labeled with a distinct detectable dye, such as the 6FAM™, TET™, VIC™, HEX™, NED™, and PET™ dyes obtainable from Applied Biosystems (Foster City, CA). For example, the DS-31 or DS-33 dye sets available from Applied Biosystems (Foster City, CA) may be used to label the primers. Each of the groups of labeled primers are added to each of the aliquots of the nucleic acids from the culture or collection of strains such that each aliquot of nucleic acid receives a single labeled primer with a single detectable dye thereon.

Each of the aliquots of nucleic acids also receives a set of unlabeled primers, with each of the unlabeled primers being complementary to a nucleotide sequence within the promoter, within a nucleotide sequence which is unique to one of the genes encoding gene products required for proliferation which were placed under the control of the replacement promoter, or within nucleotide sequences adjacent to the promoter or proliferation required genes. Each of the aliquots receives primers unique to 1/N proliferation required genes which were placed under the control of the replacement promoter, where N is the number of aliquots (i.e. if the culture or collection of strains consisted of 100 strains in which a gene required for proliferation was placed under the control of the replacement promoter and was divided into four aliquots, then each of the four aliquots of

nucleic acids from the culture or collection of strains would receive primers complementary to 25 of the genes). The unlabeled primers are selected so that each will yield an amplification product having a length distinguishable from the length of the amplification product produced with the other unlabeled primers. Preferably, the amplification products are between about 100-about 400 nucleotides in length, but any lengths which may be distinguished from each other may be used. In addition, in some of the embodiments some of the amplification products may have identical or very similar lengths but be distinguishable from one another due to labeling with distinguishable dyes.

A nucleic acid amplification reaction is conducted on each of the nucleic acid aliquots. The amplification products are then separated by length to identify amplification products decreased representation or which are absent in the culture or collection of strains. The amplification products are then correlated with the corresponding genes to determine which strains proliferated more slowly in the culture or collection of strains. If desired, amplification products having decreased representation in the culture may be identified by comparing the amplification products obtained from a culture or collection of strains which was contacted with the compound to amplification products obtained from a control culture or collection of strains which was not contacted with the compound. Alternatively, if desired, the amplification products which are missing or present at reduced levels in a culture which was contacted with the compound may be directly identified without comparison to a control culture which was not contacted with the compound.

For example, in some embodiments, the amplification products from each of the nucleic acid aliquots are pooled and subjected to capillary electrophoresis. The amplification products are detected by detecting the fluorescent dyes attached thereto and their lengths are determined to identify those amplification products having decreased representation in the culture or collection of strains. Figures 2A and 2B illustrate one embodiment of this method in which the absence of an amplification product from an amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation indicates that a test compound acts on the gene corresponding to the missing amplification product.

Alternatively, in another embodiment, a first amplification reaction is performed on nucleic acids obtained from a culture or collection of strains which was contacted with the compound using a first primer complementary to a nucleotide sequence present upstream or downstream of all of the overexpressed genes (such as a primer complementary to a nucleotide sequence in a replacement promoter upstream of all of the overexpressed genes) and a set of primers complementary to a nucleotide sequence unique to each of the strains (such as a primer complementary to a nucleotide sequence within each of the proliferation-required genes). One of the two amplification primers for each of the proliferation required genes is labeled with a dye as described above. Preferably, the common primer complementary to a nucleotide sequence upstream or downstream of all of the overexpressed genes is labeled with the dye. The primers used in the amplification reaction are designed so that the amplification product corresponding to each proliferation-required gene has a

unique length. A second amplification reaction is conducted on a control culture or collection of strains which was not contacted with the compound using the same primers as in the first amplification reaction. The amplification products from the first amplification reaction are compared to those from the second amplification reaction to identify one or more amplification products which are underrepresented in the culture or collection of strains. For example, the amplification products from the first amplification reaction may be run in a separate lane of a polyacrylamide gel or a separate capillary than the amplification products from the second amplification reaction and the two lanes or capillaries are compared to one another.

Alternatively, in some embodiments, the primers in the second amplification reaction are labeled with a different dye which is distinguishable from the dye used in the first amplification reaction. In this embodiment, the amplification reactions may be pooled and run in the same lane on a polyacrylamide gel or in the same capillary and the products from each amplification reaction are compared by comparing the amount of each dye present for each amplification product. Figures 3A and 3B illustrate one embodiment of this method in which the absence of an amplification product from the amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation which was contacted with the compound indicates that a test compound acts on the gene corresponding to the missing amplification product.

If desired, rather than dividing the culture into aliquots, individual amplification reactions may be conducted on nucleic acids obtained from the culture or collection of strains. Each amplification reaction contains primers which will yield an amplification product specific for only one of the proliferation required genes. The resulting amplification products from each of the individual amplification reactions are pooled and amplification products having decreased representation in the culture are identified as described above.

In an alternative embodiment, the representation of each strain in the culture may be assessed by hybridizing detectably labeled nucleic acids encoding the proliferation-required gene products, or portions thereof, obtained from the culture to an array comprising nucleic acids encoding the gene products required for proliferation or portions thereof. Each nucleic acid encoding a gene product required for proliferation or portion thereof occupies a known location on the array. The signal from each location on the array is quantitated to identify those nucleic acids encoding a proliferation-required gene product which are underrepresented in the culture. If desired the hybridized nucleic acids from a culture which was contacted with the compound may be compared to the hybridized nucleic acids from a control culture which was not contacted with the compound. Alternatively, the hybridized nucleic acids from a culture which was contacted with the compound may be directly analyzed without comparison to nucleic acids from a control culture.

In another alternative, each strain underexpressing a gene product required for proliferation may be constructed to contain a unique nucleic acid sequence (referred to herein as a "tag"). The tag may be included in the chromosome of each strain or in an extrachromosomal vector. For example, the tag could be included in a vector encoding an antisense nucleic acid complementary to

a gene encoding a gene product required for proliferation or a portion of such a gene or the tag may be included in the antisense nucleic acid itself. The representation of each strain in the culture may be assessed by performing an amplification reaction using primers complementary to each of the tags and quantitating the levels of the resulting amplification products to identify a tag which is underrepresented or absent from the culture. Since each tag corresponds to one strain, the strain which is underrepresented or absent from the culture may be identified. If desired the tags present in a culture which was contacted with the compound may be compared to the tags present in a control culture which was not contacted with the compound. Alternatively, the tags present in a culture which was contacted with the compound may be analyzed without comparison to a control culture.

It will be appreciated that, if desired, unique tags may also be used in embodiments in which gene products required for proliferation are overexpressed. In some aspects of such embodiments, the tags may be within or adjacent to the promoter which drives expression of the gene encoding the gene product. In such embodiments, the gene product which is overexpressed in strains which proliferate more rapidly in the culture may be identified by detecting the presence or amount of the unique tag corresponding to that gene product in the culture.

In some instances, more than one strain may proliferate less rapidly in the presence of the compound. This may result from a variety of causes. For example, the concentration of the compound may not have been high enough to reduce the proliferation only in cells which underexpress one gene product (i.e. the target gene product). While strains which underexpress the target gene product will be the least prevalent strain in the culture, other strains may also be underrepresented. In such instances, the identity of the gene product in the strain which is least prevalent in the culture (or not recovered from the culture) may be identified by quantitating the levels of each of the genes encoding proliferation-required proteins in the culture. This may be accomplished by quantitative PCR, DNA sequencing, hybridization, or array technology as described above.

In other instances, multiple strains will exhibit less rapid proliferation in the culture as a result of a common functional attribute. For example, the strains which proliferate less rapidly (or the strains which are not recovered from the culture) may each underexpress a gene product with a common enzymatic activity, such as serine protease activity for example. Alternatively, the strains which proliferate less rapidly (or the strains which are not recovered from the culture) may each underexpress a gene product with a common functional domain, such as a cAMP binding domain. In such instances, the common attribute of the strains which proliferate less rapidly (or the strains which are not recovered from the culture) may provide information as to the mode of action of the compound or the biochemical activity of the target of the compound. For example, if all of the underexpressed genes in the strains which proliferated less rapidly are serine proteases, the compound acts by inhibiting serine protease activity and the target protein is a serine protease. If desired, the compound may be derivatized and the efficacy of the derivatized compound against

each of the strains which proliferated more rapidly may be assessed as described herein in order to identify derivatives which are capable of interacting with a wide range of targets sharing a common activity or binding site (i.e. derivatives which have a greater ability to inhibit the proliferation of all the strains than the original compound) or to identify derivatives having greater specificity for a desired target (i.e. derivatives which have a greater specificity for one of the strains than the original compound).

Rather than employing a single culture which contains multiple strains each of which underexpresses a proliferation-required gene product described herein, the methods of the present invention may be performed using an array of individual strains (i.e. a collection of strains) each of which underexpresses a different proliferation-required gene product. For example, individual strains each underexpressing a different proliferation-required gene product may be grown in different wells of a multiwell plate. Each well is contacted with the compound (and, where appropriate an agent which regulates the level of expression from the promoter). The level of proliferation of the strains in each of the wells is determined to identify a strain which proliferated less rapidly or which did not proliferate at all. The identity of the underexpressed gene product in the strain that proliferated less rapidly or which did not proliferate at all is determined as described above.

In another embodiment, individual strains each underexpressing a different proliferation-required gene product (i.e. a collection of strains) are grown at different locations on a solid medium, such as an agar plate. The medium contains the compound and, where appropriate, an agent which regulates the level of expression from the promoter. The level of proliferation of each of the strains is determined to identify a strain which proliferated less rapidly (or a strain which is not recovered from the culture). The identity of the underexpressed gene product in the strain that proliferated less rapidly (or the strain which is not recovered from the culture) is determined as described above.

The above methods may be used to prioritize compound development or to determine whether the compound has been previously identified or whether the target of the compound is the target of a previously identified drug. In particular, if the product is a natural product is advantageous to determine whether it has been previously identified prior to investing significant effort in developing it. Thus, in some embodiments of the present invention, the target of a partially purified or purified natural product or a compound produced by combinatorial chemistry is identified using the methods described above and compared to the targets of known drugs. If the target is identical to that of a known drug, further development of the compound is halted.

Alternatively, an array of strains each of which underexpresses a different gene product described herein (i.e. a collection of strains) is grown on solid medium containing a compound to be evaluated. The location of each strain in the array and the gene product underexpressed by that strain is known. The pattern of colonies which grow less rapidly or fail to grow in the presenee of the compound is evaluated and compared to the pattern of colonies which grow less rapidly or fail

to grow in the presence of previously identified drugs. If the pattern of colonies which grow less rapidly or fail to grow in the presence of the compound being evaluated is the same as the pattern of colonies which grow less rapidly or fail to grow in the presence of a previously identified drug, further development of the compound is halted.

Additionally, the nucleotide sequence of the gene product described herein in a strain which proliferated less rapidly (or a strain which was not recovered from the culture) in the assays described above is compared to the nucleotide sequence of gene products from heterologous organisms to determine the likely spectrum of species whose growth would be inhibited by the compound. If the gene product has a high degree of homology to gene products from heterologous species, it is likely that the compound would also inhibit the growth of these heterologous species. Homology may be determined using any of a variety of methods familiar to those skilled in the art. For example, homology may be determined using a computer program such as BLASTP or FASTA. The ability of the compound to inhibit the growth of the heterologous species may then be confirmed by comparing the growth of cells of the heterologous species in the presence and absence of the compound.

In other embodiments, the present invention uses collections or cultures of strains comprising both strains which overexpress gene products described herein required for cellular proliferation and strains which underexpress the same gene products required for cellular proliferation. The gene product which is overexpressed or underexpressed in each strain may be a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

The culture or collection of strains is contacted with a compound and the nucleic acids present in the culture or collection of strains are analyzed. Preferably, nucleic acids derived from overexpressing strains can be distinguished from those derived from underexpressing strains. For example, the overexpressing strains may be obtained using promoter replacement as described above while the underexpressing strains may be obtained by expressing antisense nucleic acids. Accordingly, in one embodiment, amplification primers may be designed which will uniquely amplify nucleic acids from the overexpressing strains or the underexpressing strains. If a compound acts on a gene product which was overexpressed and underexpressed in the culture, then the amplification product obtained from the strain in the culture or collection which overexpressed gene product will be overrepresented in the culture or collection while the amplification product obtained from the strain which underexpressed the gene product will be underrepresented in the culture or collection. If desired, nucleic acids from a culture or collection which was contacted with

the compound may be compared to nucleic acids from a control culture or collection which was not contacted with the compound. Alternatively, nucleic acids from a culture or collection which was contacted with the compound may be directly analyzed without comparison to a control culture or collection.

In some embodiments, strains are constructed in which a nucleic acid complementary to a gene encoding a gene product described herein required for proliferation or a portion thereof is operably linked to a regulatable promoter. For example, in some embodiments, the strains may transcribe an antisense nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 or fragments thereof which inhibit proliferation or reduce the activity or level of the gene product encoded by the gene comprising a nucleotide sequence complementary to the antisense nucleic acid or homologous antisense nucleic acids or fragments thereof. In other embodiments, the strains may transcribe an antisense nucleic acid which reduces the activity or level of a gene product encoded by SEQ ID NOs.: 6214-42397, the polypeptides of SEQ ID NOs.: 42398-78581, homologous coding nucleic acids or homologous polypeptides. A culture comprising a plurality of such strains wherein each strain expresses an antisense nucleic acid against a different gene product required for proliferation is grown in the presence of varying levels of a compound which inhibits proliferation and in the presence of varying levels of an agent which regulates the level of transcription from the regulatable promoter. Nucleic acids samples are obtained from the culture, detectably labeled and hybridized to a solid support comprising nucleic acids containing the genes encoding the proliferation-required gene products or a portion thereof. The level of hybridization is quantitated for each nucleic acid encoding each of the proliferation-required gene products to determine the rate at which each of the strains proliferated in the culture. If the antisense nucleic acid expressed by a strain in the culture is not complementary to all or a portion of the gene encoding the target of the compound (i.e. a nonspecific strain), then the hybridization intensity for that strain will not be correlated with the concentration of the compound (See Figure 4), while if the antisense nucleic acid expressed by a strain in the culture is complementary to all or a portion of the gene encoding the target of the compound, the hybridization intensity for that strain will be intimately correlated with the concentration of the compound (See Figure 5). In this manner, the target of the compound may be identified. It will be appreciated that, as described above, rather than growing the strains in a single culture, each strain may be grown in a different location on a solid medium or in a different well of a multiwell plate.

The methods described above can be simultaneously performed for each of a large number of compounds. For example, the compounds may be members of a library of compounds generated using combinatorial chemistry or members of a natural product library. In such methods, a plurality of cultures each comprising a plurality of strains each of which overexpresses or underexpresses a different gene product required for proliferation or a plurality of collections of individual strains each of which overexpresses or underexpresses a different gene product required for proliferation is

obtained. Each culture or collection of strains is contacted with a different compound in the library and the target of the compound is identified as described above.

In still another embodiment, the antisense nucleic acids of the present invention (including the antisense nucelic acids of SEQ ID NOs. 1-6213 fragments thereof or homologous antisense nucleic acids or fragements thereof) that inhibit bacterial growth or proliferation can be used as antisense therapeutics for killing bacteria. The antisense sequences can be complementary to one of SEQ ID NOs.: 6214-42397 or fragments thereof, homologous coding nucleic acids or fragments thereof. Alternatively, antisense therapeutics can be complementary to operons in which proliferation-required genes reside (i.e. the antisense nucleic acid may hybridize to a nucleotide sequence of any gene in the operon in which the proliferation-required genes reside). Further, antisense therapeutics can be complementary to a proliferation-required gene or portion thereof with or without adjacent noncoding sequences, an intragenic sequence (i.e. a sequence within a gene), an intergenic sequence (i.e. a sequence between genes), a sequence spanning at least a portion of two or more genes, a 5' noncoding region or a 3' noncoding region located upstream or downstream from the actual sequence that is required for bacterial proliferation or an operon containing a proliferation-required gene.

In addition to therapeutic applications, the present invention encompasses the use of nucleic acids complementary to nucleic acids required for proliferation as diagnostic tools. For example, nucleic acid probes comprising nucleotide sequences complementary to proliferation-required sequences that are specific for particular species of cells or microorganisms can be used as probes to identify particular microorganism species or cells in clinical specimens. This utility provides a rapid and dependable method by which to identify the causative agent or agents of a bacterial infection. This utility would provide clinicians the ability to accurately identify the species responsible for the infection and amdminister a compound effective against it. In an extension of this utility, antibodies generated against proteins translated from mRNA transcribed from proliferation-required sequences can also be used to screen for specific cells or microorganisms that produce such proteins in a species-specific manner.

Other embodiments of the present invention include methods of identifying compounds which inhibit the activity of gene products required for cellular proliferation using rational drug design. As discussed in more detail below, in such methods, the structure of the gene product is determined using techniques such as x-ray crystallography or computer modeling. Compounds are screened to identify those which have a structure which would allow them to interact with the gene product or a portion thereof to inhibit its activity. The compounds may be obtained using any of a variety of methods familiar to those skilled in the art, including combinatorial chemistry. In some embodiments, the compounds may be obtained from a natural product library. In some embodiments, compounds having a structure which allows them to interact with the active site of a gene product, such as the active site of an enzyme, or with a portion of the gene product which interacts with another biomolecule to form a complex are identified. If desired, lead compounds may be identified and further optimized to provide compounds which are highly effective against the gene product.

The following examples teach the genes of the present invention and a subset of uses for the genes identified as required for proliferation. These examples are illustrative only and are not intended to limit the scope of the present invention.

EXAMPLES

The following examples are directed to the identification and exploitation of genes required for proliferation. Methods of gene identification are discussed as well as a variety of methods to utilize the identified sequences. It will be appreciated that any of the antisense nucleic acids, proliferartion-required genes or proliferation-required gene products described herein, or portions thereof, may be used in the procedures described below, including the antisense nucleic acids of SEQ ID NOs.: 1-6213, the nucleic acids of SEQ ID NOS.: 6214-42397, or the polypeptides of SEQ ID NOs.: 42398-78581. Likewise, homologous antisense nucleic acids, homologous coding nucleic acids, homologous polypeptides or portions of any of the above-mentioned nucleic acids or polypeptides, may be used in any of the procedures described below.

Genes Identified as Required for Proliferation of *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium*.

Genomic fragments were operably linked to an inducible promoter in a vector and assayed for growth inhibition activity. Example 1 describes the examination of a library of genomic fragments cloned into vectors comprising inducible promoters. Upon induction with xylose or IPTG, the vectors produced an RNA molecule corresponding to the subcloned genomic fragments. In those instances where the genomic fragments were in an antisense orientation with respect to the promoter, the transcript produced was complementary to at least a portion of an mRNA (messenger RNA) encoding a *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Salmonella typhimurium* gene product such that they interacted with sense mRNA produced from various *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Salmonella typhimurium* genes and thereby decreased the translation efficiency or the level of the sense messenger RNA thus decreasing production of the protein encoded by these sense mRNA molecules. In cases where the sense mRNA encoded a protein required for proliferation, bacterial cells containing a vector from which transcription from the promoter had been induced failed to grow or grew at a substantially reduced rate. Additionally, in cases where the transcript produced was complementary to at least a portion of a non-translated RNA and where that non-translated RNA was required for proliferation, bacterial cells containing a vector from which transcription from the promoter had been induced also failed to grow or grew at a substantially reduced rate. In contrast, cells grown under non-inducing conditions grow at a normal rate.

The above method was used to identify genes required for cellular proliferation in *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium.* Additionally, a number of genes required for cellular

proliferation in *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium*, which have been described in the following U.S. Patent Applications: U.S. Patent Application Serial Number 09/492,709, filed January 27, 2000; U.S. Patent Application Serial Number 09/711,164, filed November 9, 2000; U.S. Patent Application Serial Number 09/741,669, filed December 19, 2000 and U.S. Patent Application Serial Number 09/815,242 filed March 21, 2001, U.S. Provisional Patent Application Serial Number 60/342,923, filed October 25, 2001, have been previously identified using the above method.

EXAMPLE 1

Inhibition of Bacterial Proliferation after Induction of Antisense Expression

To identify genes required for proliferation of *E. coli*, random genomic fragments were cloned into the IPTG-inducible expression vector pLEX5BA (Krause et al., J. Mol. Biol. 274: 365 (1997) or a modified version of pLEX5BA, pLEX5BA-3' in which a synthetic linker containing a T7 terminator was ligated between the PstI and HindIII sites of pLEX5BA. In particular, to construct pLEX5BA-3', the following oligonucleotides were annealed and inserted into the PstI and HindIII sites of pLEX5BA:

5'-GTCTAGCATAACCCCTTGGGGCCTCTAAACGGGTCCTTGAGGGGTTTTTTGA-3' (SEQ ID NO: 78584)

5'-AGCTTCAAAAAACCCCTCAAGGACCCGTTTAGAGGCCCCAAGGGGTTAT GCTAGACTGCA-3' (SEQ ID NO: 78585)

Random fragments of *E. coli* genomic DNA were generated by DNAseI digestion or sonication, filled in with T4 polymerase, and cloned into the SmaI site of pLEX5BA or pLEX5BA-3'. Upon activation or induction, the promoter transcribed the random genomic fragments.

A number of vectors which allow the production of transcripts which have an extended lifetime in *E. coli* as well as other Gram negative bacteria can also be utilized in conjunction with these antisense inhibition experiments. Such vectors are described in U.S. Provisional Patent Application Serial Number 60/343,512, filed December 21, 2001. Briefly, the stabilized antisense RNA may comprise an antisense RNA which was identified as inhibiting proliferation as described above which has been engineered to contain at least one stem loop flanking each end of the antisense nucleic acid. In some embodiments, the at least one stem-loop structure formed at the 5' end of the stabilized antisense nucleic acid comprises a flush, double stranded 5' end. In some embodiments, one or more of the stem loops comprises a rho independent terminator. In additional embodiments, the stabilized antisense RNA lacks a ribosome binding site. In further embodiments, the stabilized RNA lacks sites which are cleaved by one or more RNAses, such as RNAse E or RNAse III. In some embodiments, the stabilized antisense RNA may be transcribed in a cell which the activity of at least one enzyme involved in RNA degradation has been reduced. For example, the activity of an enzyme such as RNase E, RNase II, RNase III, polynucleotide phosphorylase, and poly(A) polymerase, RNA helicase, enolase or an enzyme having similar functions may be reduced in the cell.

To study the effects of transcriptional induction in liquid medium, growth curves were carried out by back diluting cultures 1:200 into fresh media with or without 1 mM IPTG and measuring the OD_{450} every 30 minutes (min). To study the effects of transcriptional induction on solid medium, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 fold dilutions of overnight cultures were prepared. Aliquots of from 0.5 to 3 μl of these dilutions were spotted on selective agar plates with or without 1 mM IPTG. After overnight incubation, the plates were compared to assess the sensitivity of the clones to IPTG.

Of the numerous clones tested, some clones were identified as containing a sequence that inhibited *E. coli* growth after IPTG induction. Accordingly, the gene to which the inserted nucleic acid sequence corresponds, or a gene within the operon containing the inserted nucleic acid, is required for proliferation in *E. coli.*

Nucleic acids involved in proliferation of *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium* were identified as follows. Randomly generated fragments of *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Salmonella typhimurium* genomic DNA were transcribed from inducible promoters.

In the case of *Staphylococcus aureus,* a novel inducible promoter system, XylT5, comprising a modified T5 promoter fused to the *xylO* operater from the *xylA* promoter of *Staphylococcus aureus* was used. The promoter is described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001. Transcription from this hybrid promoter is inducible by xylose.

Randomly generated fragments of *Salmonella typhimurium* genomic DNA were transcribed from an IPTG inducible promoter in pLEX5BA (Krause et al., J. Mol. Biol. 274: 365 (1997) or a derivative thereof. Randomly generated fragements of *Klebsiella pneumoniae* genomic DNA were expressed from an IPTG inducible promoter in pLEX5BA-Kan. To construct pLEX5BA-kan, pLEX5BA was digested to completion with *ClaI* in order to remove the *bla* gene. Then the plasmid was treated with a partial *Not*I digestion and blunted with T4 DNA polymerase. A 3.2 kbp fragment was then gel purified and ligated to a blunted 1.3 kbp kan gene from pKanπ. Kan resistant transformants were selected on Kan plates. Orientation of the kan gene was checked by *Sma*I digestion. A clone, which had the kan gene in the same orientation as the bla gene, was used to identify genes required for proliferation of *Klebsiella pneumoniae.* Randomly generated fragments of *Pseudomonas aeruginosa* genomic DNA were trancribed from a two-component inducible promoter system. Integrated on the chromosome was the T7 RNA polymerase gene regulated by *lac*UV5/ *lac*O (Brunschwig, E. and Darzins, A. 1992. Gene 111:35-41. On a separate plasmid, a T7 gene 10 promoter, which is transcribed by T7 RNA polymerase, was fused with a *lac*O operator followed by a multiple cloning site.

Should the genomic DNA downstream of the promoter contain, in an antisense orientation, at least a portion of an mRNA or a non-translated RNA encoding a gene product involved in proliferation, then induction of transcription from the promoter will result in detectable inhibition of proliferation.

In the case of *Staphylococcus aureus,* a shotgun library of *Staphylococcus aureus* genomic fragments was cloned into the vector pXylT5-P15a, which harbors the XylT5 inducible promoter. The vector was linearized at a unique *Bam*HI site immediately downstream of the XylT5 promoter/operator. The linearized vector was treated with shrimp alkaline phosphatase to prevent reclosure of the linearized ends. Genomic DNA isolated from *Staphylococcus aureus* strain RN450 was fully digested with the restriction enzyme *Sau*3A , or , alternatively, partially digested with DNase I and "blunt-ended" by incubating with T4 DNA polymerase. Random genomic fragments between 200 and 800 base pairs in length were selected by gel purification. The size-selected genomic fragments were added to the linearized and dephosphorylated vector at a molar ratio of 0.1 to 1, and ligated to form a shotgun library.

The ligated products were transformed into electrocompetent *E. coli* strain XL1-Blue MRF' (Stratagene) and plated on LB medium with supplemented with carbenicillin at 100 μg/ml. Resulting colonies numbering 5 x 10^5 or greater were scraped and combined, and were then subjected to plasmid purification.

The purified library was then transformed into electrocompetent *Staphylococcus aureus* RN4220. Resulting transformants were plated on agar containing LB + 0.2% glucose (LBG medium) + chloramphenicol at 15 μg/ml (LBG+CM15 medium) in order to generate 100 to 150 platings at 500 colonies per plating. The colonies were subjected to robotic picking and arrayed into wells of 384 well culture dishes. Each well contained 100μl of LBG + CM15 liquid medium. Inoculated 384 well dishes were incubated 16 hours at 37°C, and each well was robotically gridded onto solid LBG + CM15 medium with or without 2% xylose. Gridded plates were incubated 16 hours at 37°C, and then manually scored for arrayed colonies that were growth-compromised in the presence of xylose.

Arrayed colonies that were growth-sensitive on medium containing 2% xylose, yet were able to grow on similar medium lacking xylose, were subjected to further growth sensitivity analysis as follows: Colonies from the plate lacking xylose were manually picked and inoculated into individual wells of a 96 well culture dish containing LBG + CM15, and were incubated for 16 hours at 37°C. These cultures were robotically diluted 1/100 into fresh medium and allowed to incubate for 4 hours at 37°C, after which they were subjected to serial dilutions in a 384 well array and then gridded onto media containing 2% xylose or media lacking xylose. After growth for 16 hours at 37°C, the arrays that resulted on the two media were compared to each other. Clones that grew similarly at all dilutions on both media were scored as a negative and were no longer considered. Clones that grew on xylose medium but failed to grow at the same serial dilution on the non-xylose plate were given a score based on the differential, i.e. should the clone grow at a

serial dilution of 10^4 or less on the xylose plate and grow at a serial dilution of 10^8 or less on the non-xylose plate, then the corresponding clone received a score of "4" representing the log difference in growth observed.

For *Salmonella typhimurium* and *Klebsiella pneumoniae* growth curves were carried out by back diluting cultures 1:200 into fresh media containing 1 mM IPTG or media lacking IPTG and measuring the OD_{450} every 30 minutes (min). To study the effects of transcriptional induction on solid medium, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7 and 10^8 fold dilutions of overnight cultures were prepared. Aliquots of from 0.5 to 3 µl of these dilutions were spotted on selective agar plates with or without 1 mM IPTG. After overnight incubation, the plates were compared to assess the sensitivity of the clones to IPTG.

Nucleic acids involved in proliferation of *Pseudomonas aeruginosa* were identified as follows. Randomly generated fragments of *Pseudomonas aeruginosa* genomic DNA were transcribed from a two-component inducible promoter system. Integrated on the chromosome was the T7 RNA polymerase gene regulated by *lac*UV5/ *lac*O (Brunschwig, E. and Darzins, A. 1992. Gene 111:35-41). On an expression plasmid there was a T7 gene 10 promoter, which is transcribed by T7 RNA polymerase, fused with a *lac*O operator followed by a multiple cloning site. Transcription from this hybrid promoter is inducible by IPTG. Should the genomic DNA downstream of the promoter contain, in an antisense orientation, at least a portion of an mRNA encoding a gene product involved in proliferation, then induction of expression from the promoter will result in detectable inhibition of proliferation.

A shotgun library of *Pseudomonas aeruginosa* genomic fragments was cloned into the vectors pEP5, pEP5S, or other similarly constructed vectors which harbor the T7*lac*O inducible promoter. The vector was linearized at a unique *Sma*I site immediately downstream of the T7*lac*O promoter/operator. The linearized vector was treated with shrimp alkaline phosphatase to prevent reclosure of the linearized ends. Genomic DNA isolated from *Pseudomonas aeruginosa* strain PAO1 was partially digested with DNase I and "blunt-ended" by incubating with T4 DNA polymerase. Random genomic fragments between 200 and 800 base pairs in length were selected by gel purification. The size-selected genomic fragments were added to the linearized and dephosphorylated vector at a molar ratio of 2 to 1, and ligated to form a shotgun library.

The ligated products were transformed into electrocompetent *E. coli* strain XL1-Blue MRF' (Stratagene) and plated on LB medium with carbenicillin at 100 µg/ml or Streptomycin 100 µg/ml. Resulting colonies numbering 5 x 10^5 or greater were scraped and combined, and were then subjected to plasmid purification.

The purified library was then transformed into electrocompetent *Pseudomonas aeruginosa* strain PAO1. Resulting transformants were plated on LB agar with carbenicillin at 100 µg/ml or Streptomycin 40 µg/ml in order to generate 100 to 150 platings at 500 colonies per plating. The colonies were subjected to robotic picking and arrayed into wells of 384 well culture dishes. Each well contained 100 µl of LB + CB 100 or Streptomycin 40 liquid medium. Inoculated 384 well

dishes were incubated 16 hours at room temperature, and each well was robotically gridded onto solid LB + CB100 or Streptomycin 40 medium with or without 1 mM IPTG. Gridded plates were incubated 16 hours at 37°C, and then manually scored for arrayed colonies that were growth-compromised in the presence of IPTG.

Arrayed colonies that were growth-sensitive on medium containing 1 mM IPTG, yet were able to grow on similar medium lacking IPTG, were subjected to further growth sensitivity analysis as follows: Colonies from the plate lacking IPTG were manually picked and inoculated into individual wells of a 96 well culture dish containing LB + CB100 or Streptomycin 40, and were incubated for 16 hours at 30°C. These cultures were robotically diluted 1/100 into fresh medium and allowed to incubate for 4 hours at 37°C, after which they were subjected to serial dilutions in a 384 well array and then gridded onto media with and without 1 mM IPTG. After growth for 16 hours at 37°C, the arrays of serially diluted spots that resulted were compared between the two media. Clones that grew similarly at all dilutions on both media were scored as a negative and were no longer considered. Clones that grew on IPTG medium but failed to grow at the same serial dilution on the non-IPTG plate were given a score based on the differential, i.e. should the clone grow at a serial dilution of 10^4 or less on the IPTG plate and grow at a serial dilution of 10^8 or less on the IPTG plate, then the corresponding clone received a score of "4" representing the log difference in growth observed.

Following the identification of those vectors that, upon induction, negatively impacted *Pseudomonas aeruginosa* growth or proliferation, the inserts or nucleic acid fragments contained in those vectors were isolated for subsequent characterization. Vectors of interest were subjected to nucleic acid sequence determination.

Nucleic acids involved in proliferation of *E. faecalis* were identified as follows. Randomly generated fragments of genomic DNA were expressed from the vectors pEPEF3 or pEPEF14, which contain the CP25 or P59 promoter, respectively, regulated by the xyl operator/repressor. These plasmids as well as other vectors useful for the expression of nucleic acids in *Enterococcus faecalis* and other Gram positive organisms are described in U.S. Patent Application Serial Number 10/032,393, filed December 21, 2001, the disclosure or which is incorportated herein by reference in its entirety. Should the genomic DNA downstream of the promoter contain, in an antisense orientation, at least a portion of a mRNA encoding a gene product involved in proliferation, then induction of expression from the promoter will result in detectable inhibition of proliferation.

A shotgun library of *E. faecalis* genomic fragments was cloned into the vector pEPEF3 or pEPEF14, which harbor xylose inducible promoters. The vector was linearized at a unique *Sma*I site immediately downstream of the promoter/operator. The linearized vector was treated with alkaline phosphatase to prevent reclosure of the linearized ends. Genomic DNA isolated from *E. faecalis* strain OG1RF was partially digested with DNase I and "blunt-ended" by incubating with T4 DNA polymerase. Random genomic fragments between 200 and 800 base pairs in length were

selected by gel purification. The size-selected genomic fragments were added to the linearized and dephosphorylated vector at a molar ratio of 2 to 1, and ligated to form a shotgun library.

The ligated products were transformed into electrocompetent *E. coli* strain TOP10 cells (Invitrogen) and plated on LB medium with erythromycin (Erm) at 150 μg/ml. Resulting colonies numbering 5 x 10^5 or greater were scraped and combined, and were then subjected to plasmid purification.

The purified library was then transformed into electrocompetent *E. faecalis* strain OG1RF. Resulting transformants were plated on Todd-Hewitt (TH) agar with erythromycin at 10 μg/ml in order to generate 100 to 150 platings at 500 colonies per plating. The colonies were subjected to robotic picking and arrayed into wells of 384 well culture dishes. Each well contained 100 μl of THB + Erm 10 μg/ml. Inoculated 384 well dishes were incubated 16 hours at room temperature, and each well was robotically gridded onto solid TH agar + Erm with or without 5% xylose. Gridded plates were incubated 16 hours at 37°C, and then manually scored for arrayed colonies that were growth-compromised in the presence of xylose.

Arrayed colonies that were growth-sensitive on medium containing 5% xylose, yet were able to grow on similar medium lacking xylose, were subjected to further growth sensitivity analysis. Colonies from the plate lacking xylose were manually picked and inoculated into individual wells of a 96 well culture dish containing THB + Erm 10, and were incubated for 16 hours at 30°C. These cultures were robotically diluted 1/100 into fresh medium and allowed to incubate for 4 hours at 37°C, after which they were subjected to serial dilution on plates containing 5% xylose or plates lacking xylose. After growth for 16 hours at 37°C, the arrays of serially diluted spots that resulted were compared between the two media. Colonies that grew similarly on both media were scored as a negative and corresponding colonies were no longer considered. Colonies on xylose medium that failed to grow to the same serial dilution compared to those on the non-xylose plate were given a score based on the differential. For example, colonies on xylose medium that only grow to a serial dilution of -4 while they were able to grow to -8 on the non-xylose plate, then the corresponding transformant colony received a score of "4" representing the log difference in growth observed.

Following the identification of those vectors that, upon induction, negatively impacted *E. faecalis* growth or proliferation, the inserts or nucleic acid fragments contained in those expression vectors were isolated for subsequent characterization. The inserts in the vectors of interest were subjected to nucleotide sequence determination.

It will be appreciated that other restriction enzymes and other endonucleases or methodologies may be used to generate random genomic fragments. In addition, random genomic fragments may be generated by mechanical shearing. Sonication and nebulization are two such techniques commonly used for mechanical shearing of DNA.

EXAMPLE 2

Nucleotide Sequence Determination of Identified Clones Transribing Nucleic Acid Fragments with Detrimental Effects on *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Salmonella typhimurium* Proliferation

Plasmids from clones that received a dilution plating score of "2" or greater were isolated to obtain the genomic DNA insert responsible for growth inhibition as follows.

The nucleotide sequences of the nucleic acid sequences which inhibited the growth of *Escherichia coli* were determined using plasmid DNA isolated using QIAPREP (Qiagen, Valencia, CA) and methods supplied by the manufacturer. The primers used for sequencing the inserts were 5' - TGTTTATCAGACCGCTT - 3' (SEQ ID NO: 78586) and 5' - ACAATTTCACACAGCCTC - 3' (SEQ ID NO: 78587). These sequences flank the polylinker in pLEX5BA.

The nucleotide sequences of the nucleic acid sequences which inhibited the growth of *Staphylococcus aureus* were determined as follows. *Staphylococcus aureus* were grown in standard laboratory media (LB or TB with 15 ug/ml Chloramphenicol to select for the plasmid). Growth was carried out at 37°C overnight in culture tubes or 2 ml deep well microtiter plates.

Lysis of *Staphylococcus aureus* was performed as follows. Cultures (2-5 ml) were centrifuged and the cell pellets resuspended in 1.5 mg/ml solution of lysostaphin (20 μl/ml of original culture) followed by addition of 250 μl of resuspension buffer (Qiagen). Alternatively, cell pellets were resuspended directly in 250 μl of resuspension buffer (Qiagen) to which 5-20 μl of a 1 mg/ml lysostaphin solution were added.

DNA was isolated using Qiagen miniprep kits or Wizard (Qiagen) miniprep kits according to the instructions provided by the manufacturer.

The genomic DNA inserts were amplified from the purified plasmids by PCR as follows.

1 μl of Qiagen purified plasmid was put into a total reaction volume of 25 μl Qiagen Hot Start PCR mix. For *Staphylococcus aureus,* the following primers were used in the PCR reaction:

pXylT5F: CAGCAGTCTGAGTTATAAAATAG (SEQ ID NO: 78588)

LexL TGTTTTATCAGACCGCTT (SEQ ID NO: 78589)

Similar methods were conducted for *Salmonella typhimurium* and *Klebsiella pneumoniae.* For *Salmonella typhimurium* and *Klebsiella pneumoniae* the following primers were used:

5' - TGTTTTATCAGACCGCTT - 3' (SEQ ID NO: 78589) and

5'-ACAATTTCACACAGCCTC-3' (SEQ ID NO: 78587)

PCR was carried out in a PE GenAmp with the following cycle times:

Step 1. 95° C 15 min

Step 2. 94° C 45 sec

Step 3. 54° C 45 sec

Step 4. 72° C 1 minute

Step 5. Return to step 2, 29 times

Step 6. 72° C 10 minutes

Step 7. 4° C hold

The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

For *Pseudomonas aeruginosa,* plasmids from transformant colonies that received a dilution plating score of "2" or greater were isolated to obtain the genomic DNA insert responsible for growth inhibition as follows. *Pseudomonas aeruginosa* were grown in standard laboratory media (LB with carbenicillin at 100 µg/ml or Streptomycin 40 µg/ml to select for the plasmid). Growth was carried out at 30°C overnight in 100 ul culture wells in microtiter plates. To amplify insert DNA 2 ul of culture were placed into 25 ul Qiagen Hot Start PCR mix. PCR reactions were in 96 well microtiter plates. For plasmid pEP5S the following primers were used in the PCR reaction:

T7L1+: GTCGGCGATATAGGCGCCAGCAACCG (SEQ ID NO: 78590)

pStrA3: ATAATCGAGCATGAGTATCATACG (SEQ ID NO: 78591)

PCR was carried out in a PE GenAmp with the following cycle times:

Step 1. 95° C 15 min

Step 2. 94° C 45 sec

Step 3. 54° C 45 sec

Step 4. 72° C 1 minute

Step 5. Return to step 2, 29 times

Step 6. 72° C 10 minutes

Step 7. 4° C hold

The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

The purified PCR products were then directly cycle sequenced with Qiagen Hot Start PCR mix. The following primers were used in the sequencing reaction:

T7/L2: ATGCGTCCGGCGTAGAGGAT (SEQ ID NO: 78592)

PCR was carried out in a PE GenAmp with the following cycle times:

Step 1. 94° C 15 min

Step 2. 96° C 10 sec

Step 3. 50° C 5 sec

Step 4. 60 C 4 min

Step 5. Return to step 2, 24 times

Step 6. 4° C hold

The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

For *E. faecalis*, plasmids from transformant colonies that received a dilution plating score of "2" or greater were isolated to obtain the genomic DNA insert responsible for growth inhibition as follows. *E. faecalis* were grown in THB 10 µg/ml Erm at 30°C overnight in 100 ul culture wells

in microtiter plates. To amplify insert DNA 2 ul of culture were placed into 25 μl Qiagen Hot Start PCR mix. PCR reactions were in 96 well microtiter plates. The following primers were used in the PCR reaction:

pXylT5: CAGCAGTCTGAGTTATAAAATAG (SEQ ID NO: 78588) and the pEP/pAK1 primer.

PCR was carried out in a PE GenAmp with the following cycle times:

Step 1. 95° C 15 min

Step 2. 94° C 45 sec

Step 3. 54° C 45 sec

Step 4. 72° C 1 minute

Step 5. Return to step 2, 29 times

Step 6. 72° C 10 minutes

Step 7. 4° C hold

The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

The purified PCR products were then directly cycle sequenced with Qiagen Hot Start PCR mix. The following primers were used in the PCR reaction:

pXylT5: CAGCAGTCTGAGTTATAAAATAG (SEQ ID NO: 78588)

PCR was carried out in a PE GenAmp with the following cycle times:

Step 1. 94° C 15 min

Step 2. 96° C 10 sec

Step 3. 50° C 5 sec

Step 4. 60° C 4 min

Step 5. Return to step 2, 24 times

Step 6. 4° C hold

The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

The amplified genomic DNA inserts from each of the above procedures were subjected to automated sequencing. Sequence identification numbers (SEQ ID NOs) and clone names for the identified inserts are listed in Table IA and discussed below.

TABLE IA

SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name
1	E3M10000001B01	1243	P33-1.C22	2485	E1M10000260G02	3727	P1M10000105C04	4969	S1M10000025G06
2	E3M10000001A02	1244	X3S107-17	2486	E1M10000260F04	3728	P1M10000105D04	4970	S1M10000025H06
3	E3M10000001B02	1245	P35-7	2487	E1M10000260A05	3729	P1M10000105C05	4971	S1M10000025H07
4	E3M10000001C02	1246	X3S118-9	2488	E1M10000260C05	3730	P1M10000105B06	4972	S1M10000025A08
5	E3M10000001D02	1247	X3S163-1	2489	E1M10000260E05	3731	P1M10000105C08	4973	S1M10000025D08
6	E3M10000001E02	1248	X3S204-7	2490	E1M10000260C07	3732	P1M10000105H08	4974	S1M10000025F08
7	E3M10000001F02	1249	X3S177-4	2491	E1M10000260G07	3733	P1M10000105D09	4975	S1M10000025H08
8	E3M10000001G02	1250	P342-3	2492	E1M10000260B08	3734	P1M10000110E01	4976	S1M10000025A09
9	E3M10000001H02	1251	SC21.1	2493	E1M10000260D08	3735	P1M10000110F01	4977	S1M10000025B09
10	E3M10000001E03	1252	SC17.1	2494	E1M10000260E08	3736	P1M10000110G01	4978	S1M10000025C09
11	E3M10000001G03	1253	SC13.1	2495	E1M10000260E09	3737	P1M10000110B02	4979	S1M10000025D09
12	E3M10000001H03	1254	MC9.6	2496	E1M10000260C10	3738	P1M10000110B03	4980	S1M10000025E09
13	E3M10000001D04	1255	Z60-P16	2497	E1M10000260D10	3739	P1M10000110F03	4981	S1M10000025F09
14	E3M10000001E04	1256	Z86-I21	2498	E1M10000260E10	3740	P1M10000110G03	4982	S1M10000025A10
15	E3M10000001F04	1257	E1M10000109A02	2499	E1M10000260G10	3741	P1M10000110D04	4983	S1M10000025C10
16	E3M10000001G04	1258	E1M10000109A11	2500	E1M10000260H10	3742	P1M10000110F04	4984	S1M10000025D10
17	E3M10000001H04	1259	E1M10000101F05	2501	E1M10000260H11	3743	P1M10000110B05	4985	S1M10000025F10
18	E3M10000001B05	1260	E1M10000101D06	2502	E1M10000260B12	3744	P1M10000110E05	4986	S1M10000025G10
19	E3M10000001D05	1261	E1M10000101A07	2503	E1M10000260D12	3745	P1M10000110B07	4987	S1M10000025H10
20	E3M10000001G05	1262	E1M10000101H07	2504	E1M10000260G12	3746	P1M10000110B08	4988	S1M10000025C11
21	E3M10000001A06	1263	E1M10000101H09	2505	E1M10000261F01	3747	P1M10000110F08	4989	S1M10000025E11
22	E3M10000001F06	1264	E1M10000101C12	2506	E1M10000261B02	3748	P1M10000110A09	4990	S1M10000025B12
23	E3M10000001B08	1265	E1M10000103B04	2507	E1M10000261H02	3749	P1M10000110E09	4991	S1M10000025F12
24	E3M10000001E08	1266	E1M10000103D11	2508	E1M10000261G04	3750	P1M10000110F09	4992	S1M10000026C01
25	E3M10000001C09	1267	E1M10000110G01	2509	E1M10000261H05	3751	P1M10000100F01	4993	S1M10000026E01
26	E3M10000001D09	1268	E1M10000110H01	2510	E1M10000261G06	3752	P1M10000098A02	4994	S1M10000026F01
27	E3M10000001E09	1269	E1M10000110E09	2511	E1M10000261H06	3753	P1M10000098B02	4995	S1M10000026G01
28	E3M10000001B10	1270	E1M10000110A12	2512	E1M10000261D08	3754	P1M10000098A03	4996	S1M10000026H01
29	E3M10000004D01	1271	E1M10000112F05	2513	E1M10000261F08	3755	P1M10000098D03	4997	S1M10000026A02
30	E3M10000004G01	1272	E1M10000113F02	2514	E1M10000261C09	3756	P1M10000098E04	4998	S1M10000026B02
31	E3M10000004D02	1273	E1M10000113A11	2515	E1M10000261H09	3757	P1M10000098G04	4999	S1M10000026H02
32	E3M10000004C03	1274	E1M10000111C03	2516	E1M10000261E10	3758	P1M10000098A05	5000	S1M10000026B03
33	E3M10000004A04	1275	E1M10000111E04	2517	E1M10000262E01	3759	P1M10000098C05	5001	S1M10000026F03

SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name
34	E3M10000004F08	1276	E1M10000111F09	2518	E1M10000262C02	3760	P1M10000098G06	5002	S1M10000026G03
35	E3M10000004D10	1277	E1M10000115H01	2519	E1M10000262E02	3761	P1M10000098H06	5003	S1M10000026H03
36	E3M10000004F10	1278	E1M10000115G02	2520	E1M10000262F02	3762	P1M10000098C07	5004	S1M10000026A04
37	E3M10000004E11	1279	E1M10000115E03	2521	E1M10000262D03	3763	P1M10000098F07	5005	S1M10000026D04
38	E3M10000004H11	1280	E1M10000115G04	2522	E1M10000262G04	3764	P1M10000098A08	5006	S1M10000026F04
39	E3M10000005B01	1281	E1M10000115C06	2523	E1M10000262C05	3765	P1M10000098G08	5007	S1M10000026G04
40	E3M10000005C01	1282	E1M10000116B01	2524	E1M10000262A06	3766	P1M10000098H09	5008	S1M10000026H04
41	E3M10000005E01	1283	E1M10000106D02	2525	E1M10000262A07	3767	P1M10000098B11	5009	S1M10000026A05
42	E3M10000005E02	1284	E1M10000106G02	2526	E1M10000262E07	3768	P1M10000098C12	5010	S1M10000026B05
43	E3M10000005C03	1285	E1M10000106E04	2527	E1M10000262E08	3769	P1M10000099D01	5011	S1M10000026D05
44	E3M10000005D03	1286	E1M10000106F05	2528	E1M10000262B10	3770	P1M10000099G03	5012	S1M10000026F05
45	E3M10000005E03	1287	E1M10000106H05	2529	E1M10000262H10	3771	P1M10000099A09	5013	S1M10000026G05
46	E3M10000005C04	1288	E1M10000106H06	2530	E1M10000262G11	3772	P1M10000099A10	5014	S1M10000026H05
47	E3M10000005D04	1289	E1M10000106A08	2531	E1M10000262D12	3773	P1M10000099E10	5015	S1M10000026A06
48	E3M10000005H04	1290	E1M10000106E09	2532	E1M10000262G12	3774	P1M10000099F10	5016	S1M10000026B06
49	E3M10000005G05	1291	E1M10000106G10	2533	E1M10000263F01	3775	P1M10000099D11	5017	S1M10000026C06
50	E3M10000005A07	1292	E1M10000106D11	2534	E1M10000263H05	3776	P1M10000106D02	5018	S1M10000026D06
51	E3M10000005F07	1293	E1M10000122B03	2535	E1M10000263C06	3777	P1M10000106F03	5019	S1M10000026F06
52	E3M10000005B08	1294	E1M10000123D05	2536	E1M10000263G06	3778	P1M10000106H03	5020	S1M10000026G06
53	E3M10000005E08	1295	E1M10000123C09	2537	E1M10000263B07	3779	P1M10000106F04	5021	S1M10000026A07
54	E3M10000005D10	1296	E1M10000123E09	2538	E1M10000263F08	3780	P1M10000106D05	5022	S1M10000026B07
55	E3M10000005F10	1297	E1M10000123H10	2539	E1M10000263A10	3781	P1M10000106E07	5023	S1M10000026C07
56	E3M10000006C01	1298	E1M10000123F11	2540	E1M10000263A11	3782	P1M10000107E02	5024	S1M10000026D07
57	E3M10000006G02	1299	E1M10000107B02	2541	E1M10000263H11	3783	P1M10000107H02	5025	S1M10000026F07
58	E3M10000006B03	1300	E1M10000107E02	2542	E1M10000263C12	3784	P1M10000107C03	5026	S1M10000026G07
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68	E3M10000007A02	1310	E1M10000118B05	2552	E1M10000264E04	3794	P1M10000108A03	5036	S1M10000026H09
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73	E3M10000007H03	1315	E1M10000119A04	2557	E1M10000264D11	3799	P1M10000108F06	5041	S1M10000026F10
74	E3M10000007C04	1316	E1M10000131H01	2558	E1M10000264F11	3800	P1M10000108G06	5042	S1M10000026G10
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78	E3M10000008H02	1320	E1M10000131C07	2562	E1M10000265A02	3804	P1M10000109D04	5046	S1M10000026C11
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86	E3M10000009E02	1328	E1M10000133A06	2570	E1M10000265E09	3812	S4M10000001C01	5054	S1M10000027G01
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102	E3M10000011D03	1344	E1M10000124E02	2586	E1M10000267B05	3828	S4M10000010B05	5070	S1M10000027E05
103	E3M10000011C07	1345	E1M10000124G03	2587	E1M10000267A07	3829	S4M10000010D07	5071	S1M10000027F05
104	E3M10000011A09	1346	E1M10000124G04	2588	E1M10000267H07	3830	S4M10000010D08	5072	S1M10000027G05
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108	E3M10000012B02	1350	E1M10000125A02	2592	E1M10000267A10	3834	S4M10000010D10	5076	S1M10000027D06
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113	E3M10000012F07	1355	E1M10000120E05	2597	E1M10000267E12	3839	S4M10000011E10	5081	S1M10000027B07
114	E3M10000012G07	1356	E1M10000120A06	2598	E1M10000268F03	3840	S4M10000011F10	5082	S1M10000027D07
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126	E3M10000013D08	1368	E1M10000121E07	2610	E1M10000268G09	3852	S4M10000016A02	5094	S1M10000027B09
127	E3M10000013E08	1369	E1M10000121D08	2611	E1M10000268E10	3853	S4M10000020F08	5095	S1M10000027C09
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136	E3M10000016A03	1378	E1M10000137C03	2620	E1M10000269B05	3862	S4M10000024G04	5104	S1M10000027B11
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139	E3M10000016G05	1381	E1M10000137B08	2623	E1M10000269A06	3865	S4M10000024G09	5107	S1M10000027G11
140	E3M10000016H05	1382	E1M10000137G09	2624	E1M10000269E07	3866	S4M10000024C11	5108	S1M10000027H11
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146	E3M10000018E01	1388	E1M10000142H03	2630	E1M10000271F02	3872	S4M10000026C01	5114	S1M10000028B02
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168	E3M10000021H11	1410	E1M10000152G03	2652	E1M10000272C08	3894	S4M10000035F02	5136	S1M10000028A06
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174	E3M10000022C05	1416	E1M10000152H08	2658	E1M10000272G09	3900	S4M10000036H11	5142	S1M10000028D07
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176	E3M10000022C06	1418	E1M10000153H03	2660	E1M10000273E01	3902	S4M10000037A08	5144	S1M10000028H07
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180	E3M10000022F08	1422	E1M10000153A09	2664	E1M10000273E05	3906	S4M10000033G05	5148	S1M10000028D08
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198	E3M10000023A07	1440	E1M10000160B09	2682	E1M10000274F09	3924	S1M10000001E09	5166	S1M10000029C03
199	E3M10000023E07	1441	E1M10000160C09	2683	E1M10000274D10	3925	S1M10000001F09	5167	S1M10000029G03
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212	E3M10000025B01	1454	E1M10000163H01	2696	E1M10000275E05	3938	S1M10000002F02	5180	S1M10000029C07
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217	E3M10000025F04	1459	E1M10000164A02	2701	E1M10000275D08	3943	S1M10000002B05	5185	S1M10000029A09
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284	E3M10000028D06	1526	E1M10000187F11	2768	E1M10000314G10	4010	S1M10000004F02	5252	S1M10000030G11
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348	E3M10000029B12	1590	E1M10000194F06	2832	E1M10000279G03	4074	S1M10000005B09	5316	S1M10000032F04
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356	E3M10000030G01	1598	E1M10000194B10	2840	E1M10000279C10	4082	S1M10000005B12	5324	S1M10000032A06
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361	E3M10000030G03	1603	E1M10000195G02	2845	E1M10000280B02	4087	S1M10000006F02	5329	S1M10000032B07
362	E3M10000030H03	1604	E1M10000195B03	2846	E1M10000280C03	4088	S1M10000006G02	5330	S1M10000032C07
363	E3M10000030B04	1605	E1M10000195G03	2847	E1M10000280C05	4089	S1M10000006A03	5331	S1M10000032D07
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368	E3M10000030A05	1610	E1M10000195A08	2852	E1M10000280A07	4094	S1M10000006G03	5336	S1M10000032D08
369	E3M10000030B05	1611	E1M10000195E09	2853	E1M10000280C07	4095	S1M10000006A04	5337	S1M10000032E08
370	E3M10000030D05	1612	E1M10000195D10	2854	E1M10000280G07	4096	S1M10000006B04	5338	S1M10000032G08
371	E3M10000030E05	1613	E1M10000195E10	2855	E1M10000280E08	4097	S1M10000006C04	5339	S1M10000032B09
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373	E3M10000030D06	1615	E1M10000195F11	2857	E1M10000280C09	4099	S1M10000006F04	5341	S1M10000032D09
374	E3M10000030F06	1616	E1M10000196B02	2858	E1M10000280H09	4100	S1M10000006G04	5342	S1M10000032E09
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389	E3M10000030G09	1631	E1M10000196A10	2873	E1M10000281G11	4115	S1M10000006E08	5357	S1M10000032C12
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391	E3M10000030D10	1633	E1M10000196D11	2875	E1M10000281F12	4117	S1M10000006B10	5359	S1M10000032F12
392	E3M10000030E10	1634	E1M10000196D12	2876	E1M10000282D01	4118	S1M10000006C10	5360	S1M10000032G12
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396	E3M10000030B11	1638	E1M10000197D04	2880	E1M10000282F03	4122	S1M10000006A12	5364	S1M10000033D02
397	E3M10000030H11	1639	E1M10000197B05	2881	E1M10000282C04	4123	S1M10000006B12	5365	S1M10000033F02
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424	E3M10000031E07	1666	E1M10000199F02	2908	E1M10000283B05	4150	S1M10000008F01	5392	S1M10000033H10
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428	E3M10000031A08	1670	E1M10000199C06	2912	E1M10000283A07	4154	S1M10000008B03	5396	S1M10000033H11
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446	E3M10000032D01	1688	E1M10000200A03	2930	E1M10000303A02	4172	S1M10000008E09	5414	S1M10000034G03
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450	E3M10000032C02	1692	E1M10000200C07	2934	E1M10000303H03	4176	S1M10000008F10	5418	S1M10000034F04
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463	E3M10000032E05	1705	E1M10000201G06	2947	E1M10000304A03	4189	S1M10000009F02	5431	S1M10000034B07
464	E3M10000032F05	1706	E1M10000201H07	2948	E1M10000304C03	4190	S1M10000009G02	5432	S1M10000034C07
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468	E3M10000032C06	1710	E1M10000201H09	2952	E1M10000304G05	4194	S1M10000009F03	5436	S1M10000034G07
469	E3M10000032D06	1711	E1M10000201A10	2953	E1M10000304A06	4195	S1M10000009G03	5437	S1M10000034H07
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485	E3M10000032H10	1727	E1M10000203C12	2969	E1M10000306C05	4211	S1M10000009A07	5453	S1M10000034F10
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684	E3M10000036D10	1926	E1M10000216D12	3168	E1M10000295H04	4410	S1M10000014C06	5652	S1M10000038B09
685	E3M10000036F10	1927	E1M10000217D02	3169	E1M10000295A07	4411	S1M10000014D06	5653	S1M10000038D09
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687	E3M10000036H10	1929	E1M10000217H02	3171	E1M10000295C07	4413	S1M10000014H06	5655	S1M10000038H09
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714	E3M10000037H05	1956	E1M10000219C01	3198	E1M10000296H07	4440	S1M10000014B12	5682	S1M10000039G03
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716	E3M10000037C06	1958	E1M10000219E05	3200	E1M10000296F08	4442	S1M10000014E12	5684	S1M10000039C04
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722	E3M10000037E07	1964	E1M10000219H07	3206	E1M10000296F12	4448	S1M10000015B02	5690	S1M10000039B06
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724	E3M10000037G07	1966	E1M10000219A09	3208	E1M10000298C01	4450	S1M10000015D02	5692	S1M10000039H06
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743	E3M10000038D01	1985	E1M10000220F06	3227	E1M10000298F11	4469	S1M10000015A06	5711	S1M10000039E10
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756	E3M10000038D04	1998	E1M10000222D02	3240	E1M10000311E07	4482	S1M10000015G08	5724	S1M10000040G01
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762	E3M10000038E05	2004	E1M10000222E12	3246	E1M10000311E11	4488	S1M10000015B10	5730	S1M10000040B03
763	E3M10000038F05	2005	E1M10000223C04	3247	E1M10000311B12	4489	S1M10000015C10	5731	S1M10000040C03
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766	E3M10000038F06	2008	E1M10000223H11	3250	E1M10000292D08	4492	S1M10000015G10	5734	S1M10000040G03
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770	E3M10000038B07	2012	E1M10000225C02	3254	E1M10000294C02	4496	S1M10000015G11	5738	S1M10000040E04
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773	E3M10000038F07	2015	E1M10000225H03	3257	E1M10000294A04	4499	S1M10000015D12	5741	S1M10000040H04
774	E3M10000038G07	2016	E1M10000225F04	3258	E1M10000294C04	4500	S1M10000015E12	5742	S1M10000040A05
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776	E3M10000038A08	2018	E1M10000225B06	3260	E1M10000294H04	4502	S1M10000016D01	5744	S1M10000040D05
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784	E3M10000038H09	2026	E1M10000226E01	3268	E1M10000294B09	4510	S1M10000016G03	5752	S1M10000040B07
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824	E3M10000039F06	2066	E1M10000232G08	3308	E1M10000309F12	4550	S1M10000016H10	5792	S1M10000041B06
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900	E3M10000041C01	2142	E1M10000237E11	3384	E1M10000289D04	4626	S1M10000018F06	5868	S1M10000043C02
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931	E3M10000041G07	2173	E1M10000239H10	3415	K1M10000015E05	4657	S1M10000018D12	5899	S1M10000043D10
932	E3M10000041A08	2174	E1M10000239G11	3416	K1M10000019D06	4658	S1M10000018E12	5900	S1M10000043E10
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939	E3M10000041A09	2181	E1M10000240C08	3423	K1M10000032E11	4665	S1M10000019F01	5907	S1M10000043A12
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967	E3M10000042G01	2209	E1M10000242E12	3451	P1M10000026H02	4693	S1M10000019C08	5935	S1M10000044A08
968	E3M10000042A02	2210	E1M10000243F03	3452	P1M10000026F04	4694	S1M10000019F08	5936	S1M10000044B08
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972	E3M10000042A03	2214	E1M10000243F06	3456	P1M10000026G09	4698	S1M10000019B09	5940	S1M10000044G08
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1004	E3M10000043A02	2246	E1M10000245B04	3488	P1M10000040E10	4730	S1M10000020B06	5972	S1M10000045E04
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1009	E3M10000043B03	2251	E1M10000245F06	3493	P1M10000042E08	4735	S1M10000020A07	5977	S1M10000045A06
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1014	E3M10000043G04	2256	E1M10000245C11	3498	P1M10000044F07	4740	S1M10000020A08	5982	S1M10000045C07
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1018	E3M10000043B06	2260	E1M10000245B12	3502	P1M10000046C09	4744	S1M10000020H08	5986	S1M10000045D08
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1040	E3M10000043G10	2282	E1M10000247E02	3524	P1M10000055A11	4766	S1M10000021G01	6008	S1M10000045E12
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1050	E3M10000044E01	2292	E1M10000247G07	3534	P1M10000062H01	4776	S1M10000021H04	6018	S1M10000046D02
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1054	E3M10000047D02	2296	E1M10000247E11	3538	P1M10000062F06	4780	S1M10000021E05	6022	S1M10000046A03
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1056	E3M10000047C08	2298	E1M10000248G01	3540	P1M10000062D07	4782	S1M10000021H05	6024	S1M10000046D03
1057	E3M10000047D08	2299	E1M10000248A02	3541	P1M10000062D08	4783	S1M10000021A06	6025	S1M10000046G03
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1062	E3M10000050C02	2304	E1M10000248G06	3546	P1M10000063F02	4788	S1M10000021G06	6030	S1M10000046E04
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1068	E3M10000050E05	2310	E1M10000249F02	3552	P1M10000064E05	4794	S1M10000021A08	6036	S1M10000046A06
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1072	E3M10000050C06	2314	E1M10000249C06	3556	P1M10000065F01	4798	S1M10000021A09	6040	S1M10000046C07
1073	E3M10000050D06	2315	E1M10000249D06	3557	P1M10000065C03	4799	S1M10000021D09	6041	S1M10000046E07
1074	E3M10000050F06	2316	E1M10000249A07	3558	P1M10000065A04	4800	S1M10000021E09	6042	S1M10000046G07
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1076	E3M10000050A07	2318	E1M10000249B08	3560	P1M10000065D06	4802	S1M10000021A10	6044	S1M10000046B08
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1081	E3M10000050H07	2323	E1M10000249E10	3565	P1M10000066A10	4807	S1M10000021F11	6049	S1M10000046A09
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1088	E3M10000050G09	2330	E1M10000250A04	3572	P1M10000067F05	4814	S1M10000022B02	6056	S1M10000046F10
1089	E3M10000050H09	2331	E1M10000250E04	3573	P1M10000067G05	4815	S1M10000022C02	6057	S1M10000046G10
1090	E3M10000050B10	2332	E1M10000250H04	3574	P1M10000067A06	4816	S1M10000022A03	6058	S1M10000046H10
1091	E3M10000051C01	2333	E1M10000250A05	3575	P1M10000067C06	4817	S1M10000022B03	6059	S1M10000046A11
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1093	E3M10000051C03	2335	E1M10000250G07	3577	P1M10000068G01	4819	S1M10000022D03	6061	S1M10000046C11
1094	E3M10000051D03	2336	E1M10000250D09	3578	P1M10000068D04	4820	S1M10000022E03	6062	S1M10000046D11
1095	E3M10000051H03	2337	E1M10000250G09	3579	P1M10000068F04	4821	S1M10000022G03	6063	S1M10000046A12
1096	E3M10000051A04	2338	E1M10000250B10	3580	P1M10000068H05	4822	S1M10000022H03	6064	S1M10000046B12
1097	E3M10000051B04	2339	E1M10000250E10	3581	P1M10000068F08	4823	S1M10000022C04	6065	S1M10000046C12
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1101	E3M10000051F05	2343	E1M10000251A02	3585	P1M10000069G06	4827	S1M10000022B05	6069	S1M10000047C01
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1103	E3M10000051D06	2345	E1M10000251F04	3587	P1M10000070E03	4829	S1M10000022E05	6071	S1M10000047G01
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1108	E3M10000051F07	2350	E1M10000251B08	3592	P1M10000070D08	4834	S1M10000022F06	6076	S1M10000047F02
1109	E3M10000051A08	2351	E1M10000251H08	3593	P1M10000070B10	4835	S1M10000022H06	6077	S1M10000047G02
1110	E3M10000051B08	2352	E1M10000251H09	3594	P1M10000070G12	4836	S1M10000022B07	6078	S1M10000047A03
1111	E3M10000051D08	2353	E1M10000251C10	3595	P1M10000071B01	4837	S1M10000022C07	6079	S1M10000047C03
1112	E3M10000051H08	2354	E1M10000251F11	3596	P1M10000071C01	4838	S1M10000022D07	6080	S1M10000047D03
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1115	E3M10000051D09	2357	E1M10000251D12	3599	P1M10000071E04	4841	S1M10000022H07	6083	S1M10000047G03
1116	E3M10000051E09	2358	E1M10000251F12	3600	P1M10000073G03	4842	S1M10000022A08	6084	S1M10000047H03
1117	E3M10000051G09	2359	E1M10000252D01	3601	P1M10000073D04	4843	S1M10000022B08	6085	S1M10000047A04
1118	E3M10000051H09	2360	E1M10000252G02	3602	P1M10000073A06	4844	S1M10000022C08	6086	S1M10000047B04
1119	E3M10000051A10	2361	E1M10000252C03	3603	P1M10000073D09	4845	S1M10000022D08	6087	S1M10000047C04
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1121	E3M10000051D10	2363	E1M10000252B04	3605	P1M10000074B01	4847	S1M10000022G08	6089	S1M10000047E04
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1128	E3M10000051F11	2370	E1M10000252H07	3612	P1M10000075B03	4854	S1M10000022D11	6096	S1M10000047D05
1129	E3M10000051G11	2371	E1M10000252A09	3613	P1M10000075A04	4855	S1M10000022F11	6097	S1M10000047E05
1130	E3M10000051F12	2372	E1M10000252E09	3614	P1M10000075C04	4856	S1M10000022H11	6098	S1M10000047F05
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1133	E3M10000050B03	2375	E1M10000252E10	3617	P1M10000076D05	4859	S1M10000022G12	6101	S1M10000047A06
1134	E3M10000050C03	2376	E1M10000252E11	3618	P1M10000076C08	4860	S1M10000023B01	6102	S1M10000047B06
1135	E3M10000050D03	2377	E1M10000252E12	3619	P1M10000076D10	4861	S1M10000023D01	6103	S1M10000047C06
1136	E3M10000050E03	2378	E1M10000253A02	3620	P1M10000077E04	4862	S1M10000023E01	6104	S1M10000047E06
1137	E3M10000050A04	2379	E1M10000253G02	3621	P1M10000077H05	4863	S1M10000023G01	6105	S1M10000047F06
1138	E3M10000050E04	2380	E1M10000253C04	3622	P1M10000077A08	4864	S1M10000023C02	6106	S1M10000047G06
1139	E3M10000050H08	2381	E1M10000253D04	3623	P1M10000077C08	4865	S1M10000023G02	6107	S1M10000047A07
1140	E3M10000052C01	2382	E1M10000253F04	3624	P1M10000096F01	4866	S1M10000023H02	6108	S1M10000047C07
1141	E3M10000052F01	2383	E1M10000253H05	3625	P1M10000096E04	4867	S1M10000023B03	6109	S1M10000047D07
1142	E3M10000052C02	2384	E1M10000253D08	3626	P1M10000096E12	4868	S1M10000023D03	6110	S1M10000047F07
1143	E3M10000052D02	2385	E1M10000253E08	3627	P1M10000097G05	4869	S1M10000023G03	6111	S1M10000047G07
1144	E3M10000052G02	2386	E1M10000253A09	3628	P1M10000059B04	4870	S1M10000023D04	6112	S1M10000047H07
1145	E3M10000052B03	2387	E1M10000253D09	3629	P1M10000059H08	4871	S1M10000023E04	6113	S1M10000047A08
1146	E3M10000052E03	2388	E1M10000253E09	3630	P1M10000059H09	4872	S1M10000023F04	6114	S1M10000047B08
1147	E3M10000052G03	2389	E1M10000253F09	3631	P1M10000059B10	4873	S1M10000023A05	6115	S1M10000047C08
1148	E3M10000052B04	2390	E1M10000253G09	3632	P1M10000059B11	4874	S1M10000023D05	6116	S1M10000047E08
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1152	E3M10000052D05	2394	E1M10000253B11	3636	P1M10000060H04	4878	S1M10000023B07	6120	S1M10000047A09
1153	E3M10000052F05	2395	E1M10000253F11	3637	P1M10000079D01	4879	S1M10000023D07	6121	S1M10000047B09
1154	E3M10000052G05	2396	E1M10000253D12	3638	P1M10000079F06	4880	S1M10000023E07	6122	S1M10000047C09
1155	E3M10000052G06	2397	E1M10000253G12	3639	P1M10000079A10	4881	S1M10000023F07	6123	S1M10000047D09
1156	E3M10000052H06	2398	E1M10000254A03	3640	P1M10000079B10	4882	S1M10000023G07	6124	S1M10000047E09
1157	E3M10000052B07	2399	E1M10000254B03	3641	P1M10000079C10	4883	S1M10000023H07	6125	S1M10000047F09
1158	E3M10000052F08	2400	E1M10000254C03	3642	P1M10000079D10	4884	S1M10000023B08	6126	S1M10000047G09
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1161	E3M10000052F10	2403	E1M10000254G05	3645	P1M10000080E04	4887	S1M10000023G08	6129	S1M10000047B10
1162	E3M10000052D11	2404	E1M10000254H05	3646	P1M10000080B06	4888	S1M10000023A09	6130	S1M10000047D10
1163	E3M10000052D12	2405	E1M10000254B06	3647	P1M10000080C06	4889	S1M10000023B09	6131	S1M10000047E10
1164	1008-H20	2406	E1M10000254A07	3648	P1M10000081G05	4890	S1M10000023D09	6132	S1M10000047F10
1165	1011-P20	2407	E1M10000254E07	3649	P1M10000081H05	4891	S1M10000023G09	6133	S1M10000047G10
1166	1053-37	2408	E1M10000254G07	3650	P1M10000081A06	4892	S1M10000023H09	6134	S1M10000047H10
1167	1010-C11	2409	E1M10000254A08	3651	P1M10000081D12	4893	S1M10000023B10	6135	S1M10000047A11
1168	1017-H1	2410	E1M10000254B09	3652	P1M10000082A02	4894	S1M10000023C10	6136	S1M10000047B11
1169	1067-16	2411	E1M10000254F10	3653	P1M10000082B04	4895	S1M10000023D10	6137	S1M10000047C11
1170	1083-27	2412	E1M10000254A11	3654	P1M10000082A05	4896	S1M10000023E10	6138	S1M10000047E11
1171	1065-12	2413	E1M10000254C11	3655	P1M10000082C05	4897	S1M10000023F10	6139	S1M10000047F11
1172	221-41	2414	E1M10000254E12	3656	P1M10000082D05	4898	S1M10000023H10	6140	S1M10000047H11
1173	B17-6.O10	2415	E1M10000255C01	3657	P1M10000082E05	4899	S1M10000023A11	6141	S1M10000047A12
1174	910-B20	2416	E1M10000255G02	3658	P1M10000083B01	4900	S1M10000023B11	6142	S1M10000047B12
1175	B18-2.N21	2417	E1M10000255H02	3659	P1M10000083A11	4901	S1M10000023C11	6143	S1M10000047C12
1176	971-B20	2418	E1M10000255A04	3660	P1M10000083B12	4902	S1M10000023E11	6144	S1M10000047D12
1177	D1-1.A15	2419	E1M10000255D05	3661	P1M10000083C12	4903	S1M10000023F11	6145	S1M10000047E12
1178	4-28.1	2420	E1M10000255F06	3662	P1M10000084D03	4904	S1M10000023G11	6146	S1M10000047F12
1179	D1-2.B13	2421	E1M10000255G06	3663	P1M10000084A04	4905	S1M10000023A12	6147	S1M10000048C01
1180	D1-2.P21	2422	E1M10000255B08	3664	P1M10000084E04	4906	S1M10000023B12	6148	S1M10000048D01
1181	Z56-D2	2423	E1M10000255D09	3665	P1M10000084F08	4907	S1M10000023C12	6149	S1M10000048G01
1182	PJMF55	2424	E1M10000255F09	3666	P1M10000084E11	4908	S1M10000023D12	6150	S1M10000048H01
1183	R1-15.A13	2425	E1M10000255B10	3667	P1M10000085D06	4909	S1M10000023F12	6151	S1M10000048A02
1184	R1-19.H1	2426	E1M10000256F01	3668	P1M10000086B01	4910	S1M10000024D01	6152	S1M10000048B02
1185	R1-55.M2	2427	E1M10000256B02	3669	P1M10000086E01	4911	S1M10000024A02	6153	S1M10000048C02

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1187	Z8-B9	2429	E1M10000256A04	3671	P1M10000086D02	4913	S1M10000024D02	6155	S1M10000048E02
1188	E1M10000007B04	2430	E1M10000256C05	3672	P1M10000086E05	4914	S1M10000024F02	6156	S1M10000048F02
1189	227-10	2431	E1M10000256E07	3673	P1M10000087E04	4915	S1M10000024H02	6157	S1M10000048G02
1190	709-F23	2432	E1M10000256E09	3674	P1M10000087F04	4916	S1M10000024D03	6158	S1M10000048H02
1191	801-C15	2433	E1M10000256A10	3675	P1M10000087C09	4917	S1M10000024E03	6159	S1M10000048A03
1192	801-H19	2434	E1M10000256F10	3676	P1M10000087F09	4918	S1M10000024F03	6160	S1M10000048B03
1193	804-P6	2435	E1M10000256C12	3677	P1M10000087A11	4919	S1M10000024A04	6161	S1M10000048C03
1194	807-D20	2436	E1M10000257C01	3678	P1M10000088C04	4920	S1M10000024C04	6162	S1M10000048E03
1195	B13-17.G8	2437	E1M10000257G01	3679	P1M10000088A07	4921	S1M10000024D04	6163	S1M10000048F03
1196	B5-6.C8	2438	E1M10000257A02	3680	P1M10000089G08	4922	S1M10000024H04	6164	S1M10000048G03
1197	B8-2.D9	2439	E1M10000257D02	3681	P1M10000089D11	4923	S1M10000024B05	6165	S1M10000048H03
1198	B15-8.P13	2440	E1M10000257H02	3682	P1M10000090E01	4924	S1M10000024E05	6166	S1M10000048E04
1199	T13-5.A2	2441	E1M10000257C03	3683	P1M10000090F06	4925	S1M10000024F05	6167	S1M10000048G04
1200	T12-3.I11	2442	E1M10000257F04	3684	P1M10000090F08	4926	S1M10000024G05	6168	S1M10000048H04
1201	T20-15.D4	2443	E1M10000257G04	3685	P1M10000090B11	4927	S1M10000024B06	6169	S1M10000048A05
1202	T24-15.G6	2444	E1M10000257B05	3686	P1M10000091A09	4928	S1M10000024E06	6170	S1M10000048B05
1203	T24-17.C6	2445	E1M10000257D05	3687	P1M10000091E09	4929	S1M10000024G06	6171	S1M10000048C05
1204	244.B12	2446	E1M10000257F06	3688	P1M10000091G10	4930	S1M10000024H06	6172	S1M10000048F05
1205	1042-J1	2447	E1M10000257G07	3689	P1M10000092B02	4931	S1M10000024A07	6173	S1M10000048G05
1206	195.F5	2448	E1M10000257H07	3690	P1M10000092E02	4932	S1M10000024C07	6174	S1M10000048H05
1207	25.D5	2449	E1M10000257H08	3691	P1M10000092B04	4933	S1M10000024E07	6175	S1M10000048A06
1208	25.D6	2450	E1M10000257A09	3692	P1M10000092F05	4934	S1M10000024G07	6176	S1M10000048B06
1209	177.F3	2451	E1M10000257D09	3693	P1M10000092F06	4935	S1M10000024H07	6177	S1M10000048C06
1210	525.H11	2452	E1M10000257G10	3694	P1M10000092D09	4936	S1M10000024A08	6178	S1M10000048E06
1211	632.N2	2453	E1M10000257H10	3695	P1M10000092B10	4937	S1M10000024B08	6179	S1M10000048A07
1212	633.B7	2454	E1M10000257A11	3696	P1M10000092B12	4938	S1M10000024E08	6180	S1M10000048C07
1213	671.I20	2455	E1M10000257C11	3697	P1M10000093A03	4939	S1M10000024F08	6181	S1M10000048E07
1214	676.B12	2456	E1M10000257F11	3698	P1M10000093B03	4940	S1M10000024G08	6182	S1M10000048F07
1215	643.B19	2457	E1M10000257B12	3699	P1M10000093F03	4941	S1M10000024H08	6183	S1M10000048G07
1216	720.O16	2458	E1M10000257F12	3700	P1M10000093H07	4942	S1M10000024B09	6184	S1M10000048H07
1217	666.H12	2459	E1M10000258C01	3701	P1M10000093C08	4943	S1M10000024B10	6185	S1M10000048B08
1218	98.D4	2460	E1M10000258H02	3702	P1M10000093B09	4944	S1M10000024D10	6186	S1M10000048C08
1219	844.B21	2461	E1M10000258G03	3703	P1M10000093E09	4945	S1M10000024F10	6187	S1M10000048D08
1220	P31-25-F3	2462	E1M10000258A04	3704	P1M10000094H03	4946	S1M10000024G10	6188	S1M10000048E08
1221	P335-8.H8	2463	E1M10000258C04	3705	P1M10000094F04	4947	S1M10000024A11	6189	S1M10000048F08

SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name	SeqID	Clone Name
1222	P347.2	2464	E1M10000258G04	3706	P1M10000094H04	4948	S1M10000024D11	6190	S1M10000048H08
1223	P31-11-J20	2465	E1M10000258C05	3707	P1M10000094A10	4949	S1M10000024G12	6191	S1M10000048A09
1224	P336-14.F20	2466	E1M10000258D05	3708	P1M10000095C01	4950	S1M10000025B01	6192	S1M10000048C09
1225	P31-27-M1	2467	E1M10000258F05	3709	P1M10000095E04	4951	S1M10000025C01	6193	S1M10000048D09
1226	P338-4.M21	2468	E1M10000258G05	3710	P1M10000095G04	4952	S1M10000025D01	6194	S1M10000048E09
1227	P334-8.L7	2469	E1M10000258A06	3711	P1M10000095C09	4953	S1M10000025E01	6195	S1M10000048F09
1228	P31-2-E16	2470	E1M10000258D06	3712	P1M10000102E05	4954	S1M10000025B02	6196	S1M10000048H09
1229	P335-3.J14	2471	E1M10000258B07	3713	P1M10000102B07	4955	S1M10000025A03	6197	S1M10000048A10
1230	P334-5.H2	2472	E1M10000258G07	3714	P1M10000103B05	4956	S1M10000025B03	6198	S1M10000048B10
1231	P31-33-N2	2473	E1M10000258G08	3715	P1M10000103D06	4957	S1M10000025C03	6199	S1M10000048C10
1232	P332-11.C20	2474	E1M10000258B09	3716	P1M10000103E08	4958	S1M10000025D03	6200	S1M10000048D10
1233	869.A23	2475	E1M10000258D09	3717	P1M10000104A02	4959	S1M10000025F03	6201	S1M10000048E10
1234	P317-2.A3	2476	E1M10000258F10	3718	P1M10000104H02	4960	S1M10000025D04	6202	S1M10000048G10
1235	P326-9.K2	2477	E1M10000258C11	3719	P1M10000104A03	4961	S1M10000025E04	6203	S1M10000048H10
1236	P323-8.P1	2478	E1M10000258F11	3720	P1M10000104E03	4962	S1M10000025G04	6204	S1M10000048A11
1237	P35-8	2479	E1M10000259C03	3721	P1M10000104F07	4963	S1M10000025B05	6205	S1M10000048C11
1238	P36-13.E2	2480	E1M10000259B04	3722	P1M10000104D11	4964	S1M10000025C05	6206	S1M10000048D11
1239	P38-1.G20	2481	E1M10000259E04	3723	P1M10000105D01	4965	S1M10000025F05	6207	S1M10000048F11
1240	P327-50.M10	2482	E1M10000259E05	3724	P1M10000105E02	4966	S1M10000025H05	6208	S1M10000048G11
1241	P328-8.D21	2483	E1M10000259B12	3725	P1M10000105C03	4967	S1M10000025B06	6209	S1M10000048H11
1242	P328-20.P20	2484	E1M10000260E02	3726	P1M10000105G03	4968	S1M10000025D06	6210	S1M10000048A12

EXAMPLE 3

Comparison Of Isolated Nucleic Acids to Known Sequences

The nucleotide sequences of the subcloned fragments from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Salmonella typhimurium* obtained from the expression vectors discussed above were compared to known sequences from *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium* and other microorganisms as follows. First, to confirm that each clone originated from one location on the chromosome and was not chimeric, the nucleotide sequences of the selected clones were compared against the *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Salmonella typhimurium* genomic sequences to align the clone to the correct position on the chromosome. The NCBI BLASTN v 2.0.9 program was used for this comparison, and the incomplete *Staphylococcus aureus* genomic sequences licensed from TIGR, as well as the NCBI nonredundant GenBank database were used as the source of genomic data. *Salmonella typhimurium* sequences were compared to sequences available from the Genome Sequencing Center (http://genome.wustl.edu/gsc/salmonella.shtml), and the Sanger Centre (http://www.sanger.ac.uk/projects/S___typhi). *Pseudomonas aeruginosa* sequences were compared to a proprietary database and the NCBI GenBank database. The *E. faecalis* sequences were compared to a proprietary database.

The BLASTN analysis was performed using the default parameters except that the filtering was turned off. No further analysis was performed on inserts which resulted from the ligation of multiple fragments.

In general, antisense molecules and their complementary genes are identified as follows. First, all possible full length open reading frames (ORFs) are extracted from available genomic databases. Such databases include the GenBank nonredundant (nr) database, the unfinished genome database available from TIGR and the PathoSeq database developed by Incyte Genomics. The latter database comprises over 40 annotated bacterial genomes including complete ORF analysis. If databases are incomplete with regard to the bacterial genome of interest, it is not necessary to extract all ORFs in the genome but only to extract the ORFs within the portions of the available genomic sequences which are complementary to the clones of interest. Computer algorithms for identifying ORFs, such as GeneMark, are available and well known to those in the art. Comparison of the clone DNA to the complementary ORF(s) allows determination of whether the clone is a sense or antisense clone. Furthermore, each ORF extracted from the database can be compared to sequences in well annotated databases including the GenBank (nr) protein database, SWISSPROT and the like. A description of the gene or of a closely related gene in a closely related microorganism is often available in these databases. Similar methods are used to identify antisense clones corresponding to genes encoding non-translated RNAs.

In order to generate the gene identification data compiled in Table IB, each of the cloned nucleic acid sequences discussed above corresponding to SEQ ID NO.s 1-6213 was used to identify the corresponding *Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa* or *Salmonella typhimurium* ORFs in the PathoSeq v.4.1 (March 2000 release) database of microbial genomic sequences. For this purpose, the NCBI BLASTN 2.0.9 computer algorithm was used. The default parameters were used except that filtering was turned off. The default parameters for the BLASTN and BLASTX analyses were:

Expectation value (e)=10

Alignment view options: pairwise
Filter query sequence (DUST with BLASTN, SEG with others)=T
Cost to open a gap (zero invokes behavior)=0
Cost to extend a gap (zero invokes behavior)=0
X dropoff value for gapped alignment (in bits) (zero invokes behavior)=0
Show GI's in deflines=F
Penalty for a nucleotide mismatch (BLASTN only)=!3
Reward for a nucleotide match (BLASTN only)=1
Number of one-line descriptions (V)=500
Number of alignments to show (B)=250
Threshold for extending hits=default
Perform gapped alignment (not available with BLASTX)=T
Query Genetic code to use=1
DB Genetic code (for TBLAST[nx] only=1
Number of processors to use=1
SeqAlign file
Believe the query define=F
Matrix=BLOSUM62
Word Size= default
Effective length of the database (use zero for the real size)=0
Number of best hits from a region to keep=100
Length of region used to judge hits=20
Effective length of the search space (use zero for the real size)=0
Query strands to search against database (for BLAST[nx] and TBLASTX), 3 is both, 1 is top, 2 is bottom=3
Produce HTML output=F

Alternatively, ORFs were identified and refined by conducting a survey of the public and private data sources. Full-length gene protein and nucleotide sequences for these organisms were assembled from various sources. For *Pseudomonas aeruginosa*, gene sequences were adopted from the Pseudomonas genome sequencing project (downloaded from http://www.pseudomonas.com). For *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae* and *Salmonella typhi*, genomic sequences from PathoSeq v 4.1 (Mar 2000 release) was reanalyzed for ORFs using the gene finding software GeneMark v 2.4a, which was purchased from GenePro Inc. 451 Bishop St., N.W., Suite B, Atlanta, GA, 30318, USA.

Antisense clones were identified as those clones for which transcription from the inducible promoter would result in the expression of an RNA antisense to a complementary ORF, intergenic

or intragenic sequence. Those clones containing single inserts and that caused growth sensitivity upon induction are listed in Table IA.

The gene descriptions in the PathoSeq database derive from annotations available in the public sequence databases described above. Where a clone was found to share significant sequence identity to two or more adjacent ORFs, it was listed once for each ORF and the PathoSeq information for each ORF was compiled in Table IB.

Table IA lists the SEQ ID NOs. and clone names of the inserts which inhibited proliferation. This information was used to identify the ORFs (SEQ ID NOs.: 6214-42397) whose gene products (SEQ ID NOs. 42398-78581) were inhibited by the nucleic acids comprising the nucleotide sequences of SEQ ID NOs. 1-6213. Table IB lists the clone name and the PathoSeq Locus containing the clone.

TABLE IB

Clone Name	Gene LocusID	Clone Name	Gene LocusID	Clone Name	Gene LocusID
E3M10000001B01	EFA205257	E1M10000233C05	ECO103161	S1M10000005E05	SAU802496
E3M10000001B01	EFA205258	E1M10000233H05	ECO103224	S1M10000005C06	SAU802121
E3M10000001A02	EFA205257	E1M10000233H05	ECO103225	S1M10000005D06	SAU801183
E3M10000001A02	EFA205258	E1M10000233D08	ECO103185	S1M10000005D06	SAU801184
E3M10000001B02	EFA205225	E1M10000233F08	ECO103265	S1M10000005A07	SAU800967
E3M10000001B02	EFA201977	E1M10000233F08	ECO103266	S1M10000005B07	SAU802496
E3M10000001B02	EFA203137	E1M10000233A09	ECO104092	S1M10000005D07	SAU801264
E3M10000001C02	EFA200840	E1M10000233A09	ECO104093	S1M10000005A08	SAU802496
E3M10000001D02	EFA202003	E1M10000233E09	ECO103238	S1M10000005B08	SAU800548
E3M10000001E02	EFA200840	E1M10000233E09	ECO103239	S1M10000005D08	SAU800607
E3M10000001F02	EFA200807	E1M10000233F09	ECO103886	S1M10000005E08	SAU802496
E3M10000001G02	EFA205257	E1M10000233D10	ECO103242	S1M10000005B09	SAU800122
E3M10000001G02	EFA205258	E1M10000233D10	ECO103243	S1M10000005C09	SAU801481
E3M10000001H02	EFA200811	E1M10000233H10	ECO100094	S1M10000005D09	SAU800542
E3M10000001E03	EFA201987	E1M10000234E01	ECO103884	S1M10000005A10	SAU801723
E3M10000001E03	EFA205258	E1M10000234B02	ECO103886	S1M10000005A10	SAU801722
E3M10000001G03	EFA201987	E1M10000234G02	ECO103233	S1M10000005A11	SAU801644
E3M10000001G03	EFA205258	E1M10000234G02	ECO103234	S1M10000005C11	SAU801113
E3M10000001H03	EFA201987	E1M10000234C05	ECO103181	S1M10000005D11	SAU800547
E3M10000001H03	EFA205258	E1M10000234C07	ECO103844	S1M10000005E11	SAU800155
E3M10000001D04	EFA201980	E1M10000234C08	ECO103878	S1M10000005B12	SAU802160
E3M10000001D04	EFA201981	E1M10000234C08	ECO204942	S1M10000005B12	SAU603460
E3M10000001D04	EFA205229	E1M10000234F08	ECO103461	S1M10000005D12	SAU801644
E3M10000001E04	EFA201028	E1M10000234H08	ECO103226	S1M10000006F01	SAU801264
E3M10000001F04	EFA200811	E1M10000234F09	ECO103055	S1M10000006B02	SAU800381
E3M10000001G04	EFA201993	E1M10000234D10	ECO100876	S1M10000006E02	SAU802496
E3M10000001H04	EFA201980	E1M10000234G10	ECO100886	S1M10000006F02	SAU802160
E3M10000001H04	EFA201981	E1M10000234B12	ECO104010	S1M10000006G02	SAU802125
E3M10000001H04	EFA205229	E1M10000235D01	ECO102233	S1M10000006A03	SAU802496
E3M10000001B05	EFA201993	E1M10000235A03	ECO100798	S1M10000006B03	SAU802655
E3M10000001D05	EFA201974	E1M10000235H03	ECO103886	S1M10000006D03	SAU801740
E3M10000001D05	EFA201975	E1M10000235E04	ECO103236	S1M10000006E03	SAU801256
E3M10000001G05	EFA202001	E1M10000235B06	ECO103886	S1M10000006F03	SAU801434
E3M10000001G05	EFA202003	E1M10000235F06	ECO103481	S1M10000006G03	SAU801275
E3M10000001A06	EFA201028	E1M10000235B08	ECO103885	S1M10000006A04	SAU801139
E3M10000001F06	EFA201028	E1M10000235E08	ECO103161	S1M10000006B04	SAU802496
E3M10000001B08	EFA201028	E1M10000235B09	ECO101848	S1M10000006C04	SAU802158
E3M10000001E08	EFA200807	E1M10000235H09	ECO103481	S1M10000006E04	SAU801089
E3M10000001C09	EFA200839	E1M10000235H09	ECO103482	S1M10000006F04	SAU801644
E3M10000001D09	EFA201987	E1M10000235B10	ECO100886	S1M10000006G04	SAU801740
E3M10000001D09	EFA205258	E1M10000235A11	ECO102299	S1M10000006A05	SAU802224
E3M10000001E09	EFA201987	E1M10000235F12	ECO103233	S1M10000006A05	SAU802223
E3M10000001E09	EFA205258	E1M10000235F12	ECO103234	S1M10000006D05	SAU802496
E3M10000001B10	EFA205257	E1M10000236E01	ECO100095	S1M10000006G05	SAU801256
E3M10000001B10	EFA205258	E1M10000236A02	ECO102340	S1M10000006C06	SAU800331
E3M10000004D01	EFA201985	E1M10000236E02	ECO103878	S1M10000006C06	SAU800332
E3M10000004D01	EFA201984	E1M10000236E02	ECO204942	S1M10000006D06	SAU802496
E3M10000004D01	EFA202953	E1M10000236A03	ECO103287	S1M10000006F06	SAU800548
E3M10000004G01	EFA200839	E1M10000236D03	ECO102556	S1M10000006G06	SAU800006
E3M10000004D02	EFA202022	E1M10000236G03	ECO102655	S1M10000006A07	SAU800967
E3M10000004D02	EFA202028	E1M10000236A04	ECO103186	S1M10000006B07	SAU801760

Clone Name	Gene LocusID	Clone Name	Gene LocusID	Clone Name	Gene LocusID
E3M10000004D02	EFA202536	E1M10000236D04	ECO103481	S1M10000006C07	SAU800546
E3M10000004C03	EFA200412	E1M10000236G04	ECO103510	S1M10000006D07	SAU801105
E3M10000004A04	EFA201981	E1M10000236A05	ECO102847	S1M10000006E07	SAU802496
E3M10000004A04	EFA205229	E1M10000236F05	ECO103181	S1M10000006G07	SAU801731
E3M10000004F08	EFA201977	E1M10000236F05	ECO103182	S1M10000006A08	SAU802496
E3M10000004F08	EFA203137	E1M10000236H06	ECO103242	S1M10000006E08	SAU802238
E3M10000004D10	EFA201999	E1M10000236H06	ECO103243	S1M10000006A10	SAU802496
E3M10000004D10	EFA201997	E1M10000236D08	ECO103669	S1M10000006B10	SAU802240
E3M10000004F10	EFA200624	E1M10000236F09	ECO103228	S1M10000006C10	SAU802496
E3M10000004E11	EFA200624	E1M10000236C10	ECO102227	S1M10000006G10	SAU802247
E3M10000004H11	EFA205225	E1M10000236A11	ECO102986	S1M10000006G10	SAU802248
E3M10000004H11	EFA201977	E1M10000236C11	ECO101088	S1M10000006B11	SAU801618
E3M10000004H11	EFA203137	E1M10000236F12	ECO101355	S1M10000006G11	SAU802119
E3M10000005B01	EFA201984	E1M10000237A02	ECO103161	S1M10000006G11	SAU802118
E3M10000005B01	EFA201983	E1M10000237B02	ECO101830	S1M10000006A12	SAU800548
E3M10000005C01	EFA200839	E1M10000237E04	ECO103217	S1M10000006B12	SAU802558
E3M10000005E01	EFA201977	E1M10000237E04	ECO103218	S1M10000007F01	SAU801256
E3M10000005E01	EFA203137	E1M10000237H04	ECO103624	S1M10000007B02	SAU800591
E3M10000005E02	EFA201977	E1M10000237H04	ECO103625	S1M10000007B02	SAU800592
E3M10000005E02	EFA203137	E1M10000237G06	ECO103232	S1M10000007F02	SAU801366
E3M10000005C03	EFA200811	E1M10000237G06	ECO103233	S1M10000007G02	SAU801138
E3M10000005C03	EFA200812	E1M10000237C07	ECO103886	S1M10000007A03	SAU801899
E3M10000005D03	EFA200811	E1M10000237G07	ECO103263	S1M10000007D03	SAU802496
E3M10000005D03	EFA200812	E1M10000237H07	ECO102267	S1M10000007G03	SAU800967
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E3M10000005E03	EFA200812	E1M10000237A08	ECO103216	S1M10000007E04	SAU802496
E3M10000005C04	EFA200660	E1M10000237B08	ECO101185	S1M10000007F04	SAU800478
E3M10000005C04	EFA200661	E1M10000237B08	ECO101186	S1M10000007C05	SAU800547
E3M10000005D04	EFA200839	E1M10000237D08	ECO103217	S1M10000007G05	SAU800548
E3M10000005H04	EFA200839	E1M10000237D08	ECO103216	S1M10000007C06	SAU801900
E3M10000005G05	EFA201977	E1M10000237E08	ECO103262	S1M10000007D06	SAU800547
E3M10000005G05	EFA203137	E1M10000237E08	ECO103878	S1M10000007E06	SAU801113
E3M10000005A07	EFA200811	E1M10000237E08	ECO204942	S1M10000007C07	SAU801904
E3M10000005A07	EFA200812	E1M10000237B09	ECO101844	S1M10000007E07	SAU801618
E3M10000005F07	EFA200839	E1M10000237D10	ECO102060	S1M10000007G07	SAU802638
E3M10000005B08	EFA201977	E1M10000237D10	ECO102061	S1M10000007C08	SAU800482
E3M10000005B08	EFA203137	E1M10000237E11	ECO100169	S1M10000007E08	SAU800700
E3M10000005E08	EFA202276	E1M10000238G01	ECO103451	S1M10000007F08	SAU802261
E3M10000005D10	EFA201977	E1M10000238A02	ECO103514	S1M10000007F09	SAU800210
E3M10000005D10	EFA203137	E1M10000238F03	ECO101939	S1M10000007D10	SAU800537
E3M10000005F10	EFA201977	E1M10000238B04	ECO103262	S1M10000007F10	SAU802240
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E3M10000006C01	EFA201981	E1M10000238D04	ECO104147	S1M10000007D11	SAU801900
E3M10000006G02	EFA202214	E1M10000238F04	ECO103224	S1M10000008F01	SAU802160
E3M10000006G02	EFA202216	E1M10000238F04	ECO103225	S1M10000008F01	SAU603460
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E3M10000006B03	EFA201997	E1M10000238F05	ECO100194	S1M10000008G02	SAU802643
E3M10000006D03	EFA201982	E1M10000238F05	ECO100195	S1M10000008A03	SAU802177
E3M10000006D03	EFA201981	E1M10000238D06	ECO101185	S1M10000008A03	SAU802176
E3M10000006F04	EFA200811	E1M10000238D06	ECO101186	S1M10000008B03	SAU800023
E3M10000006F04	EFA200812	E1M10000238F06	ECO103229	S1M10000008F03	SAU800753
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E3M10000050D05	EFA202953	E1M10000287A10	ECO103237	S1M10000028A02	SAU801769
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E3M10000052G02	EFA200457	E1M10000291B04	ECO103238	S1M10000029C10	SAU800529
E3M10000052B03	EFA200239	E1M10000291E04	ECO100967	S1M10000029D10	SAU801790
E3M10000052E03	EFA202378	E1M10000291E05	ECO103223	S1M10000029E10	SAU801139
E3M10000052G03	EFA200326	E1M10000291G05	ECO102555	S1M10000029F10	SAU800266
E3M10000052B04	EFA200290	E1M10000291G05	ECO102556	S1M10000029H10	SAU801139

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E3M10000052D05	EFA201888	E1M10000291E07	ECO103866	S1M10000029C12	SAU802070
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E3M10000052G06	EFA202274	E1M10000291B08	ECO103218	S1M10000029F12	SAU800257
E3M10000052H06	EFA200805	E1M10000291F08	ECO103229	S1M10000029G12	SAU801138
E3M10000052B07	EFA202168	E1M10000291B10	ECO103881	S1M10000030B01	SAU802654
E3M10000052F08	EFA202378	E1M10000291E10	ECO101591	S1M10000030D01	SAU801113
E3M10000052E09	EFA201985	E1M10000291D11	ECO103263	S1M10000030F01	SAU801473
E3M10000052E09	EFA202953	E1M10000291F11	ECO100095	S1M10000030H01	SAU800543
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E3M10000052F10	EFA200240	E1M10000291H11	ECO103262	S1M10000030C02	SAU802567
E3M10000052D11	EFA200898	E1M10000291H11	ECO103878	S1M10000030D02	SAU801515
E3M10000052D12	EFA202168	E1M10000291H11	ECO204942	S1M10000030E02	SAU801473
1008-H20	ECO100023	E1M10000291B12	ECO103882	S1M10000030H02	SAU802452
1011-P20	ECO100702	E1M10000291F12	ECO103243	S1M10000030B03	SAU802654
1053-37	ECO101256	E1M10000293B01	ECO103885	S1M10000030C03	SAU800275
1053-37	ECO202228	E1M10000293B02	ECO104093	S1M10000030D03	SAU801473
1010-C11	ECO101324	E1M10000293G02	ECO103886	S1M10000030G03	SAU800542
1017-H1	ECO304472	E1M10000293A04	ECO100402	S1M10000030H03	SAU800232
1067-16	ECO102309	E1M10000293B04	ECO103886	S1M10000030C04	SAU800526
1083-27	ECO102636	E1M10000293A05	ECO100095	S1M10000030A05	SAU800478
1065-12	ECO102557	E1M10000293E05	ECO103223	S1M10000030B05	SAU801256
221-41	ECO103884	E1M10000293E05	ECO103224	S1M10000030C05	SAU800526
B17-6.O10	ECO103884	E1M10000293G05	ECO103243	S1M10000030D05	SAU800759
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B18-2.N21	ECO100148	E1M10000293H06	ECO102654	S1M10000030G05	SAU800776
971-B20	ECO103240	E1M10000293F07	ECO101095	S1M10000030G05	SAU800777
971-B20	ECO103241	E1M10000293C08	ECO101844	S1M10000030H05	SAU800179
D1-1.A15	ECO103394	E1M10000293E08	ECO101939	S1M10000030D06	SAU800189
4-28.1	ECO101485	E1M10000293G08	ECO103101	S1M10000030E06	SAU801257
D1-2.B13	ECO102255	E1M10000293B09	ECO103181	S1M10000030B07	SAU802627
D1-2.P21	ECO102144	E1M10000293G09	ECO102144	S1M10000030D07	SAU800189
Z56-D2	ECO103911	E1M10000293H09	ECO100094	S1M10000030G07	SAU802247
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PJMF55	ECO103265	E1M10000293A11	ECO103883	S1M10000030C08	SAU801831
R1-15.A13	ECO101995	E1M10000293E11	ECO103242	S1M10000030F08	SAU802231
R1-19.H1	ECO101104	E1M10000293F11	ECO104091	S1M10000030F08	SAU802230
R1-55.M2	ECO103884	E1M10000293F11	ECO104092	S1M10000030G08	SAU802250
Z45-F11	ECO103263	E1M10000293C12	ECO100170	S1M10000030A09	SAU801719
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709-F23	ECO101506	E1M10000295G01	ECO103532	S1M10000030F09	SAU801904
801-C15	ECO100488	E1M10000295G01	ECO103533	S1M10000030G09	SAU800542
801-C15	ECO100490	E1M10000295B02	ECO101635	S1M10000030H09	SAU801644
801-C15	ECO100491	E1M10000295E02	ECO103217	S1M10000030A10	SAU802309
801-H19	ECO100488	E1M10000295E02	ECO103218	S1M10000030A10	SAU802308
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804-P6	ECO102513	E1M10000295A07	ECO100712	S1M10000030F10	SAU800019
807-D20	ECO100366	E1M10000295B07	ECO100179	S1M10000030G10	SAU800019
807-D20	ECO100367	E1M10000295B07	ECO100180	S1M10000030H10	SAU802654
B13-17.G8	ECO101111	E1M10000295C07	ECO103224	S1M10000030A11	SAU800517
B5-6.C8	ECO101475	E1M10000295C07	ECO103225	S1M10000030A11	SAU202623
B5-6.C8	ECO101476	E1M10000295C07	ECO103226	S1M10000030D11	SAU800517
B5-6.C8	ECO201962	E1M10000295D08	ECO103225	S1M10000030D11	SAU202623
B8-2.D9	ECO103461	E1M10000295D08	ECO103226	S1M10000030E11	SAU802241
B15-8.P13	ECO101328	E1M10000295F08	ECO103160	S1M10000030G11	SAU800811
B15-8.P13	ECO101329	E1M10000295G08	ECO103217	S1M10000030C12	SAU801647
T13-5.A2	ECO103059	E1M10000295G08	ECO103218	S1M10000030C12	SAU801646
T12-3.I11	ECO102857	E1M10000295B09	ECO103236	S1M10000030E12	SAU800537
T20-15.D4	ECO101475	E1M10000295F09	ECO103881	S1M10000030G12	SAU801526
T20-15.D4	ECO101476	E1M10000295F09	ECO103882	S1M10000031B01	SAU802240
T20-15.D4	ECO201962	E1M10000295G09	ECO103263	S1M10000031H01	SAU800023
T24-15.G6	ECO103059	E1M10000295D10	ECO103101	S1M10000031B02	SAU802247
T24-17.C6	ECO102857	E1M10000295H10	ECO103263	S1M10000031E02	SAU801912
244.B12	ECO101763	E1M10000295B11	ECO103229	S1M10000031F02	SAU802231
244.B12	ECO101764	E1M10000295F11	ECO100954	S1M10000031F02	SAU802230
244.B12	ECO101765	E1M10000295G12	ECO103494	S1M10000031G02	SAU802235
1042-J1	ECO100702	E1M10000312D11	ECO104091	S1M10000031G02	SAU802234
1042-J1	ECO100703	E1M10000312D11	ECO104092	S1M10000031H02	SAU801355
195.F5	ECO102842	E1M10000296B01	ECO102304	S1M10000031A03	SAU802250
25.D5	ECO103059	E1M10000296C02	ECO102466	S1M10000031E03	SAU801134
25.D6	ECO103059	E1M10000296C02	ECO102467	S1M10000031E03	SAU801135
177.F3	ECO102309	E1M10000296D02	ECO103235	S1M10000031F03	SAU802240
525.H11	ECO102857	E1M10000296D02	ECO103236	S1M10000031G03	SAU801505
632.N2	ECO104277	E1M10000296D02	ECO103237	S1M10000031A04	SAU801434
633.B7	ECO103479	E1M10000296H02	ECO102556	S1M10000031A04	SAU302892
671.I20	ECO103478	E1M10000296C03	ECO100150	S1M10000031B04	SAU800543
676.B12	ECO103479	E1M10000296C03	ECO100151	S1M10000031C04	SAU800738
643.B19	ECO100702	E1M10000296E03	ECO101086	S1M10000031C04	SAU800737
720.O16	ECO103884	E1M10000296H03	ECO103227	S1M10000031E04	SAU800542
666.H12	ECO103478	E1M10000296H03	ECO103228	S1M10000031F04	SAU801517
666.H12	ECO103479	E1M10000296D04	ECO103237	S1M10000031F04	SAU801516
98.D4	ECO103263	E1M10000296G04	ECO102144	S1M10000031G04	SAU302611
844.B21	ECO102144	E1M10000296F05	ECO103886	S1M10000031G04	SAU302882
P31-25-F3	ECO101686	E1M10000296G05	ECO101467	S1M10000031F05	SAU800548
P335-8.H8	ECO101041	E1M10000296H05	ECO103094	S1M10000031D06	SAU801526
P347.2	ECO101086	E1M10000296A06	ECO100194	S1M10000031G06	SAU800548
P31-11-J20	ECO103228	E1M10000296A06	ECO100195	S1M10000031H06	SAU600582
P336-14.F20	ECO101370	E1M10000296G07	ECO102827	S1M10000031C07	SAU801760
P31-27-M1	ECO103423	E1M10000296G07	ECO102828	S1M10000031D07	SAU801181
P338-4.M21	ECO100139	E1M10000296H07	ECO103220	S1M10000031E07	SAU800016
P334-8.L7	ECO101256	E1M10000296H07	ECO103221	S1M10000031A08	SAU802365
P31-2-E16	ECO101686	E1M10000296E08	ECO100886	S1M10000031D08	SAU801790
P335-3.J14	ECO100523	E1M10000296F08	ECO103218	S1M10000031E08	SAU800547
P334-5.H2	ECO100139	E1M10000296G08	ECO103734	S1M10000031F08	SAU801264
P31-33-N2	ECO103241	E1M10000296H08	ECO100809	S1M10000031C09	SAU801193
P332-11.C20	ECO102827	E1M10000296H08	ECO100810	S1M10000031D09	SAU800019
P332-11.C20	ECO102828	E1M10000296A09	ECO100194	S1M10000031G09	SAU800006
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P326-9.K2	ECO103293	E1M10000296F12	ECO101684	S1M10000031E10	SAU800001
P323-8.P1	ECO101685	E1M10000296G12	ECO100095	S1M10000031F10	SAU800244
P35-8	ECO103692	E1M10000298C01	ECO101438	S1M10000031G10	SAU800962
P36-13.E2	ECO103059	E1M10000298G01	ECO104148	S1M10000031A11	SAU801741
P38-1.G20	ECO102227	E1M10000298G01	ECO104149	S1M10000031B11	SAU801908
P327-50.M10	ECO103242	E1M10000298G02	ECO102636	S1M10000031C11	SAU802152
P327-50.M10	ECO103243	E1M10000298C03	ECO103238	S1M10000031F11	SAU800312
P328-8.D21	ECO103240	E1M10000298C03	ECO103239	S1M10000031G11	SAU801234
P328-8.D21	ECO103241	E1M10000298D03	ECO103886	S1M10000031H11	SAU800962
P328-20.P20	ECO100541	E1M10000298H03	ECO103262	S1M10000031B12	SAU801621
P33-1.C22	ECO103227	E1M10000298H03	ECO103878	S1M10000031C12	SAU801741
X3S107-17	ECO101475	E1M10000298H03	ECO204942	S1M10000031E12	SAU801275
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P35-7	ECO103928	E1M10000298H04	ECO100809	S1M10000032C01	SAU800548
X3S118-9	ECO103263	E1M10000298H04	ECO100808	S1M10000032F01	SAU800525
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SC13.1	ECO101348	E1M10000298G06	ECO100096	S1M10000032C03	SAU800771
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E1M10000220B03	ECO101324	S1M10000002D08	SAU801900	S1M10000047G05	SAU801669
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E1M10000220B05	ECO103242	S1M10000002C09	SAU800542	S1M10000047C06	SAU802217
E1M10000220E05	ECO101257	S1M10000002E09	SAU800547	S1M10000047E06	SAU802224
E1M10000220H05	ECO100171	S1M10000002F09	SAU801481	S1M10000047F06	SAU800543
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E1M10000222F05	ECO101476	S1M10000003C06	SAU800014	S1M10000047A10	SAU801301
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E1M10000223H11	ECO102802	S1M10000003A09	SAU800700	S1M10000047C11	SAU801434
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E1M10000225E02	ECO100009	S1M10000003A11	SAU801113	S1M10000047D12	SAU801831
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E1M10000225H03	ECO101066	S1M10000003B12	SAU801434	S1M10000047F12	SAU802223
E1M10000225F04	ECO101583	S1M10000003B12	SAU302892	S1M10000048C01	SAU800753
E1M10000225A06	ECO103218	S1M10000003C12	SAU800548	S1M10000048D01	SAU802654
E1M10000225B06	ECO101259	S1M10000003F12	SAU801621	S1M10000048G01	SAU800363
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E1M10000225H09	ECO101953	S1M10000004F01	SAU800966	S1M10000048C02	SAU800490
E1M10000225H09	ECO101989	S1M10000004G01	SAU802496	S1M10000048D02	SAU802548
E1M10000225F10	ECO101684	S1M10000004C02	SAU802496	S1M10000048D02	SAU104011
E1M10000225D12	ECO102966	S1M10000004F02	SAU800546	S1M10000048E02	SAU800753
E1M10000225D12	ECO102967	S1M10000004B03	SAU800257	S1M10000048F02	SAU802107
E1M10000225G12	ECO101128	S1M10000004B03	SAU800258	S1M10000048G02	SAU800019
E1M10000226E01	ECO100469	S1M10000004D03	SAU800187	S1M10000048G02	SAU800020
E1M10000226B02	ECO100683	S1M10000004E03	SAU800153	S1M10000048H02	SAU800547
E1M10000226B02	ECO103405	S1M10000004G03	SAU800016	S1M10000048A03	SAU801630
E1M10000226B02	ECO103515	S1M10000004A04	SAU802655	S1M10000048B03	SAU801184
E1M10000226B02	ECO204900	S1M10000004B04	SAU801760	S1M10000048C03	SAU802586
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E1M10000226F02	ECO102999	S1M10000004D04	SAU802223	S1M10000048E03	SAU802586
E1M10000226D03	ECO103160	S1M10000004E04	SAU802247	S1M10000048E03	SAU802585
E1M10000226G03	ECO103696	S1M10000004G05	SAU800548	S1M10000048F03	SAU802226
E1M10000226F04	ECO102508	S1M10000004A06	SAU801644	S1M10000048G03	SAU800543
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E1M10000226H06	ECO103848	S1M10000004D06	SAU800006	S1M10000048E04	SAU801183
E1M10000226A08	ECO104132	S1M10000004E06	SAU802240	S1M10000048G04	SAU802247
E1M10000226D08	ECO101753	S1M10000004F06	SAU800152	S1M10000048H04	SAU802586
E1M10000226D09	ECO100430	S1M10000004A07	SAU802503	S1M10000048H04	SAU802585
E1M10000226D09	ECO100431	S1M10000004D07	SAU802496	S1M10000048A05	SAU801263
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E1M10000226D10	ECO101623	S1M10000004E07	SAU802176	S1M10000048C05	SAU801891
E1M10000226E10	ECO102714	S1M10000004F07	SAU801683	S1M10000048F05	SAU801184
E1M10000226G11	ECO103244	S1M10000004G07	SAU801644	S1M10000048G05	SAU800542
E1M10000226B12	ECO101916	S1M10000004G07	SAU801643	S1M10000048H05	SAU800546
E1M10000226F12	ECO100240	S1M10000004B08	SAU801346	S1M10000048A06	SAU800546
E1M10000227E03	ECO100975	S1M10000004B08	SAU200535	S1M10000048B06	SAU801184
E1M10000227E03	ECO201249	S1M10000004C08	SAU802503	S1M10000048C06	SAU801670
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E1M10000227G03	ECO101459	S1M10000004D08	SAU202623	S1M10000048E06	SAU801186
E1M10000227E04	ECO104148	S1M10000004F08	SAU802224	S1M10000048A07	SAU801253
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E1M10000227B08	ECO102736	S1M10000004C10	SAU801742	S1M10000048B08	SAU800018
E1M10000227B08	ECO102737	S1M10000004C10	SAU801741	S1M10000048C08	SAU800453
E1M10000227D08	ECO103911	S1M10000004D10	SAU801618	S1M10000048D08	SAU300619
E1M10000227B09	ECO102736	S1M10000004A11	SAU801475	S1M10000048E08	SAU802224
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E1M10000227E11	ECO103723	S1M10000004A12	SAU801208	S1M10000048A09	SAU800547
E1M10000227E11	ECO103724	S1M10000004C12	SAU800519	S1M10000048C09	SAU800548
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E1M10000227D12	ECO104144	S1M10000004E12	SAU800528	S1M10000048F09	SAU802238
E1M10000232H02	ECO103235	S1M10000004F12	SAU801900	S1M10000048H09	SAU800546

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E1M10000232B03	ECO101324	S1M10000005C01	SAU802496	S1M10000048C10	SAU800367
E1M10000232H03	ECO103097	S1M10000005E01	SAU800996	S1M10000048D10	SAU802590
E1M10000232C07	ECO100170	S1M10000005B02	SAU802243	S1M10000048E10	SAU802590
E1M10000232F07	ECO103797	S1M10000005D02	SAU800519	S1M10000048G10	SAU802238
E1M10000232F07	ECO103798	S1M10000005E02	SAU802655	S1M10000048H10	SAU802240
E1M10000232G07	ECO104010	S1M10000005F02	SAU801644	S1M10000048A11	SAU802224
E1M10000232A08	ECO100850	S1M10000005F02	SAU801643	S1M10000048C11	SAU802217
E1M10000232G08	ECO100875	S1M10000005A03	SAU802310	S1M10000048D11	SAU802090
E1M10000232G12	ECO102636	S1M10000005D03	SAU800548	S1M10000048F11	SAU802496
E1M10000233C01	ECO103886	S1M10000005F03	SAU802262	S1M10000048G11	SAU801186
E1M10000233A03	ECO100784	S1M10000005B04	SAU801183	S1M10000048H11	SAU801139
E1M10000233B03	ECO100784	S1M10000005D04	SAU801183	S1M10000048A12	SAU801139
E1M10000233D03	ECO100118	S1M10000005D04	SAU801184	S1M10000048B12	SAU802502
E1M10000233H03	ECO103238	S1M10000005F04	SAU800363	S1M10000048D12	SAU800250
E1M10000233H03	ECO103239	S1M10000005F04	SAU800362	S1M10000048D12	SAU800249
E1M10000233C04	ECO102309	S1M10000005F04	SAU800361	S1M10000048G12	SAU802251
E1M10000233G04	ECO101185	S1M10000005C05	SAU801264		
E1M10000233A05	ECO102553	S1M10000005D05	SAU801644		

Table IC provides a cross reference between PathoSeq Gene Loci listed in Table IB and the SEQ ID NOs. of the corresponding PathoSeq polypeptides and the SEQ ID NOs. of the nucleic acids which encode them. The Gene Locus IDs provided in Table IC each comprise a nine digit alpha-numeric identifier that can be used to determine the organism from which each Gene Locus and corresponding SEQ ID NOs. were identified. Specifically, the first letter of the Gene Locus ID corresponds to the first letter of the genus name of the organism described herein from which the Gene Locus was identified and the second and third letters of the Gene Locus ID correspond to the first two letters of the species name of this organism. For example, the identifier EFA205257 describes a gene locus identified from *Enterococcus faecalis*. In those instances where the three letter identifier is the same for different organisms, the exact identity of the organism which corresponds to the Gene Locus ID can be determined by referring to the organism designation in the sequence listing for the coding nucleic acid or polypeptide SEQ ID NO. that corresponds to the particular Gene Locus ID.

TABLE IC

DNA SeqID	Protein SeqID	Gene LocusID	DNA SeqID	Protein SeqID	Gene LocusID	DNA SeqID	Protein SeqID	Gene LocusID
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6215	42399	EFA205258	18277	54461	CJU100848	30338	66522	PAE203654
6216	42400	EFA205225	18278	54462	CJU100855	30339	66523	PAE203656
6217	42401	EFA201977	18279	54463	CJU100856	30340	66524	PAE203658
6218	42402	EFA203137	18280	54464	CJU100859	30341	66525	PAE203668
6219	42403	EFA200840	18281	54465	CJU100860	30342	66526	PAE203670
6220	42404	EFA202003	18282	54466	CJU100861	30343	66527	PAE203672
6221	42405	EFA200807	18283	54467	CJU100862	30344	66528	PAE203677
6222	42406	EFA200811	18284	54468	CJU100863	30345	66529	PAE203684
6223	42407	EFA201987	18285	54469	CJU100866	30346	66530	PAE203691
6224	42408	EFA201980	18286	54470	CJU100870	30347	66531	PAE203698
6225	42409	EFA201981	18287	54471	CJU100871	30348	66532	PAE203722
6226	42410	EFA205229	18288	54472	CJU100872	30349	66533	PAE203732
6227	42411	EFA201028	18289	54473	CJU100885	30350	66534	PAE203735
6228	42412	EFA201993	18290	54474	CJU100886	30351	66535	PAE203739
6229	42413	EFA201974	18291	54475	CJU100888	30352	66536	PAE203740
6230	42414	EFA201975	18292	54476	CJU100890	30353	66537	PAE203741
6231	42415	EFA202001	18293	54477	CJU100891	30354	66538	PAE203742
6232	42416	EFA200839	18294	54478	CJU100896	30355	66539	PAE203743
6233	42417	EFA201985	18295	54479	CJU100903	30356	66540	PAE203744
6234	42418	EFA201984	18296	54480	CJU100923	30357	66541	PAE203751
6235	42419	EFA202953	18297	54481	CJU100925	30358	66542	PAE203754
6236	42420	EFA202022	18298	54482	CJU100929	30359	66543	PAE203755
6237	42421	EFA202028	18299	54483	CJU100938	30360	66544	PAE203757
6238	42422	EFA202536	18300	54484	CJU100944	30361	66545	PAE203758
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6240	42424	EFA201999	18302	54486	CJU100955	30363	66547	PAE203766
6241	42425	EFA201997	18303	54487	CJU100961	30364	66548	PAE203774
6242	42426	EFA200624	18304	54488	CJU100965	30365	66549	PAE203796
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6244	42428	EFA200812	18306	54490	CJU100967	30367	66551	PAE203799
6245	42429	EFA200660	18307	54491	CJU100970	30368	66552	PAE203800
6246	42430	EFA200661	18308	54492	CJU100980	30369	66553	PAE203801
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6248	42432	EFA201982	18310	54494	CJU100983	30371	66555	PAE203812
6249	42433	EFA202214	18311	54495	CJU100987	30372	66556	PAE203813
6250	42434	EFA202216	18312	54496	CJU100989	30373	66557	PAE203820
6251	42435	EFA200360	18313	54497	CJU100993	30374	66558	PAE203821
6252	42436	EFA200766	18314	54498	CJU100996	30375	66559	PAE203828
6253	42437	EFA200805	18315	54499	CJU100997	30376	66560	PAE203829
6254	42438	EFA201637	18316	54500	CJU100998	30377	66561	PAE203831
6255	42439	EFA201986	18317	54501	CJU100999	30378	66562	PAE203850
6256	42440	EFA205255	18318	54502	CJU101006	30379	66563	PAE203869
6257	42441	EFA200495	18319	54503	CJU101007	30380	66564	PAE203871
6258	42442	EFA104836	18320	54504	CJU101011	30381	66565	PAE203872
6259	42443	EFA201976	18321	54505	CJU101017	30382	66566	PAE203887
6260	42444	EFA201523	18322	54506	CJU101021	30383	66567	PAE203888
6261	42445	EFA202012	18323	54507	CJU101022	30384	66568	PAE203892
6262	42446	EFA202007	18324	54508	CJU101027	30385	66569	PAE203897
6263	42447	EFA200307	18325	54509	CJU101028	30386	66570	PAE203898
6264	42448	EFA201888	18326	54510	CJU101029	30387	66571	PAE203900
6265	42449	EFA205285	18327	54511	CJU101031	30388	66572	PAE203911

DNA SeqID	Protein SeqID	Gene LocusID	DNA SeqID	Protein SeqID	Gene LocusID	DNA SeqID	Protein SeqID	Gene LocusID
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